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MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

FIELD OF THE INVENTION

The invention generally pertains to the field of manufacture of fatty acids, particularly for large-scale production of very long chain polyunsaturated fatty acids (VLC-PUFAs, also called polyunsaturated fatty acids or PUFAs), e.g. eicosapentaenoic acid (EPA), omega-3 docosapentaenoic acid (DPA) and docosahexaenoic acid (DHA). The invention particularly is concerned with the production of VLC-PUFAs in plants and thus inter alia provides nucleic acids for transformation of plants to enable such transformed plants to produce VLC-PUFAs. To this end, the invention also provides transgenic constructs and expression vectors containing desaturase and elongase genes and host cells into which the constructs and expression vectors have been introduced. The present invention also relates to methods for the manufacture of oil, fatty acid- or lipids-containing compositions, and to such oils and lipids as such. In addition, the invention is concerned with methods for further improving the production of VLC-PUFAs in plants.

BACKGROUND OF THE INVENTION

Fatty acids are carboxylic acids with long-chain hydrocarbon side groups that play a fundamental role in many biological processes. Fatty acids are rarely found free in nature but, rather, occur in esterified form as the major component of lipids. As such, lipids/fatty acids are sources of energy (e.g., beta-oxidation). In addition, lipids/fatty acids are an integral part of cell membranes and, therefore, are indispensable for processing biological or biochemical information.

Long chain polyunsaturated fatty acids (VLC-PUFAs) such as docosahexaenoic acid (DHA, 22:6(4,7,10,13,16,19)) are essential components of cell membranes of various tissues and organelles in mammals (nerve, retina, brain and immune cells). Clinical studies have shown that DHA is essential for the growth and development of the brain in infants, and for maintenance of normal brain function in adults (Martinetz, M. (1992) J. Pediatr. 120:S129 S138). DHA also has significant effects on photoreceptor function involved in the signal transduction process, rhodopsin activation, and rod and cone development (Giusto, N.M., et al. (2000) Prog. Lipid Res. 39:315-391). In addition, some positive effects of DHA were also found on diseases such as hypertension, arthritis, atherosclerosis, depression, thrombosis and cancers (Horrocks, L.A. and Yeo, Y.K. (1999) Pharmacol. Res. 40:211-215). Therefore, appropriate dietary supply of the fatty acid is important for human health. Because such fatty acids cannot be efficiently synthesized by infants, young children and senior citizens, it is particularly important for these individuals to adequately intake these fatty acids from the diet (Spector, A.A. (1999) Lipids 34:S1 S3).

EPA and also ARA (arachidonic acid, 20:4n-6 (5,8,11,14)) are both delta 5 (d5) essential fatty acids. They form a unique class of food and feed constituents for humans and animals. EPA belongs to the n-3 series with five double bonds in the acyl chain. EPA is found in marine food and is abundant in oily fish from North Atlantic. ARA belongs to the n-6 series with four double bonds. The lack of a double bond in the delta-3 position confers on ARA different properties than those found in EPA. The eicosanoids produced from ARA (sometimes abbreviated "AA") have strong inflammatory and platelet aggregating properties, whereas those derived from EPA have anti-inflammatory and anti-platelet aggregating properties. ARA can be obtained from some foods such as meat, fish and eggs, but the concentration is low.

Gamma-linolenic acid (GLA, C18:3n-6 (6,9,12)) is another essential fatty acid found in mammals. GLA is the metabolic intermediate for very long chain n-6 fatty acids and for various active molecules. In mammals, formation of long chain polyunsaturated fatty acids is rate-limited by delta-6 desaturation. Many physiological and pathological conditions such as aging, stress, diabetes, eczema, and some infections have been shown to depress the delta-6 desaturation step. In addition, GLA is readily catabolized from the oxidation and rapid cell division associated with certain disorders, e.g., cancer or inflammation. Therefore, dietary supplementation with GLA can reduce the risks of these disorders. Clinical studies have shown that dietary supplementation with GLA is effective in treating some pathological conditions such as atopic eczema, premenstrual syndrome, diabetes, hypercholesterolemia, and inflammatory and cardiovascular disorders.

A large number of benefitial health effects have been shown for DHA or mixtures of EPA and DHA.

Although biotechnology offers an attractive route for the production of specialty fatty acids, current techniques fail to provide an efficient means for the large scale production of unsaturated fatty acids. Accordingly, there exists a need for an improved and efficient method of producing very long chain poly unsaturated fatty acids (VLC-PUFAs), such as EPA and DHA.

To make possible the fortification of food and/or of feed with polyunsaturated omega-3-fatty acids, there is still a great need for a simple, inexpensive process for the production of each of the aforementioned long chain polyunsaturated fatty acids, especially in eukaryotic systems.

SUMMARY OF THE INVENTION

The invention is thus concerned with providing a reliable source for easy manufacture of VLC-PUFAs. To this end the invention is also concerned with providing plants reliably

producing VLC-PUFAS, preferably EPA and/or DHA. The invention is also concerned with providing means and methods for obtaining, improving and farming such plants, and also with VLC-PUFA containing oil obtainable from such plants, particularly from the seeds thereof. Also, the invention provides uses for such plants and parts thereof.

According to the invention there is thus provided a T-DNA for expression of a target gene in a plant. The invention beneficially provides a system for transformation of plant tissue and for generation of recombinant plants, wherein the recombinant plant differs from the respective parental plant (for the purposes of the present invention the parental plant is termed a wild-type plant regardless of whether or not such parental plant is as such found in nature) by the introduction of T-DNA. The T-DNA introduced into the parental plant beneficially has a length of at least 30000 nucleotides.

The invention also provides plants with a genotype that confers a heritable phenotype of high seed oil VLC-PUFA content in one or more of their tissues or components, preferably a high content of EPA and/or DHA in seed oil. The invention further provides material comprising a high VLC-PUFA content relative to their total oil content, preferably a high content of EPA and/or DHA. Also, the invention provides exemplary events of Brassica plants. Most beneficially the invention provides oil comprising a high VLC-PUFA content, preferably a high content of EPA and/or DHA.

The invention also provides methods of producing an oil, wherein the oil has a high VLC-PUFA content, a high content of EPA and/or DHA. In particularly preferred aspects these methods are for producing a corresponding plant oil.

The invention also provides methods for creating a plant, such that the plant or progeny thereof can be used as a source of an oil, wherein the oil has a high VLC-PUFA content, a high content of EPA and/or DHA. Thus, the invention beneficially also provides methods for the production of plants having a heritable phenotype of high seed oil VLC-PUFA content in one or more of their tissues or components, preferably a high content of EPA and/or DHA in seed oil.

The invention also provides means for optimizing a method for creating plants according to the invention. In this respect, the invention provides a system of methods for analyzing enzyme specificities, particularly for analyzing desaturase reaction specificity and for analyzing elongase specificities, for optimization of a metabolic pathway and for determining CoA-dependence of a target desaturase.

DETAILED DESCRIPTION OF THE INVENTION

Various aspects of the invention are hereinafter described in more detail. It is to be understood that the detailed description is not intended to limit the scope of the claims.

The term "polyunsaturated fatty acids (PUFA)" as used herein refers to fatty acids comprising at least two, preferably, three, four, five or six, double bonds. Moreover, it is to be understood that such fatty acids comprise, preferably from 18 to 24 carbon atoms in the fatty acid chain. More preferably, the term relates to long chain PUFA (VLC-PUFA) having from 20 to 24 carbon atoms in the fatty acid chain. Particularly, polyunsaturated fatty acids in the sense of the present invention are DHGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), iARA 20:4(8,11,14,17), EPA 20:5 (5,8,11,14,17), DPA 22:5 (4,7,10,13,16), DHA 22:6 (4,7,10,13,16,19), 20:4 (8,11,14,17), more preferably, arachidonic acid (ARA) 20:4 (5,8,11,14), eicosapentaenoic acid (EPA) 20:5 (5,8,11,14,17), and docosahexaenoic acid (DHA) 22:6 (4,7,10,13,16,19). Thus, it will be understood that most preferably, the methods provided by the present invention pertaining to the manufacture of EPA and/or DHA. Moreover, also encompassed are the intermediates of VLC-PUFA which occur during synthesis. Such intermediates are, preferably, formed from substrates by the desaturase, keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity of the polypeptide of the present invention. Preferably, substrates encompass LA 18:2 (9,12), GLA 18:3 (6,9,12), DHGLA 20:3 (8,11,14), ARA 20:4 (5,8,11,14), eicosadienoic acid 20:2 (11,14), eicosatetraenoic acid 20:4 (8,11,14,17), eicosapentaenoic acid 20:5 (5,8,11,14,17). Systematic names of fatty acids including polyunsaturated fatty acids, their corresponding trivial names and shorthand notations used according to the present invention are given in table Table 18 (and in table 181).

The term "cultivating" as used herein refers to maintaining and growing the transgenic plant under culture conditions which allow the cells to produce the said polyunsaturated fatty acid, i.e. the PUFA and/or VLC-PUFA referred to above. This implies that the polynucleotide of the present invention is expressed in the transgenic plant so that the desaturase, elongase as also the keto-acyl-CoA-synthase, keto-acyl-CoA-reductase, dehydratase and enoyl-CoA-reductase activity is present. Suitable culture conditions for cultivating the host cell are described in more detail below.

The term "obtaining" as used herein encompasses the provision of the cell culture including the host cells and the culture medium or the plant or plant part, particularly the seed, of the current invention, as well as the provision of purified or partially purified preparations thereof comprising the polyunsaturated fatty acids, preferably, ARA, EPA, DHA, in free or in CoA bound form, as membrane phospholipids or as triacylglyceride estres. More preferably, the PUFA and VLC-PUFA are to be obtained as triglyceride esters, e.g., in form of an oil. More details on purification techniques can be found elsewhere herein below.

The term "polynucleotide" according to the present invention refers to a desoxyribonucleic acid or ribonucleic acid. Unless stated otherwise, "polynucleotide" herein refers to a single strand of a DNA polynucleotide or to a double stranded DNA polynucleotide. The length of a polynucleotide is designated according to the invention by the specification of a number of basebairs ("bp") or nucleotides ("nt"). According to the invention, both specifications are used interchangeably, regardless whether or not the respective nucleic acid is a single or double stranded nucleic acid. Also, as polynucleotides are defined by their respective nucleotide sequence, the terms nucleotide/polynucleotide and nucleotide sequence/polynucleotide sequence are used interchangeably, thus that a reference to a nucleic acid sequence also is meant to define a nucleic acid comprising or consisting of a nucleic acid stretch the sequence of which is identical to the nucleic acid sequence.

In particular, the term "polynucleotide" as used in accordance with the present invention as far as it relates to a desaturase or elongase gene relates to a polynucleotide comprising a nucleic acid sequence which encodes a polypeptide having desaturase or elongase activity. Preferably, the polypeptide encoded by the polynucleotide of the present invention having desaturase, or elongase activity upon expression in a plant shall be capable of increasing the amount of PUFA and, in particular, VLC-PUFA in, e.g., seed oils or an entire plant or parts thereof. Whether an increase is statistically significant can be determined by statistical tests well known in the art including, e.g., Student's t-test with a confidentiality level of at least 90%, preferably of at least 95% and even more preferably of at least 98%. More preferably, the increase is an increase of the amount of triglycerides containing VLC-PUFA of at least 5%, at least 10%, at least 15%, at least 20% or at least 30% compared to wildtype control. Preferably, the VLC-PUFA referred to before is a polyunsaturated fatty acid having a C20, C22 or C24 fatty acid body, more preferably EPA or DHA. Lipid analysis of oil samples are shown in the accompanying Examples.

The fatty acid esters with polyunsaturated C20- and/or C22-fatty acid molecules can be isolated in the form of an oil or lipid, for example, in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetylcoenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three, four, five or six, preferably five or six, double bonds, from the organisms which were used for the preparation of the fatty acid esters. Preferably, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine, especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the non-human transgenic organisms or host cells, preferably in the plants, as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty acids) are present

in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight. In the process of the invention, the VLC-PUFAs which have been produced are produced in a content as for DHA of at least 5,5% by weight, at least 6% by weight, at least 7% by weight, advantageously at least 8% by weight, preferably at least 9% by weight, especially preferably at least 10,5% by weight, very especially preferably at least 20% by weight, as for EPA of at least 9,5% by weight, at least 10% by weight, at least 11% by weight, advantageously at least 12% by weight, preferably at least 13% by weight, especially preferably at least 14,5% by weight, very especially preferably at least 30% by weight based on the total fatty acids in the non-human transgenic organisms or the host cell referred to above. The fatty acids are, preferably, produced in bound form. It is possible, with the aid of the polynucleotides and polypeptides of the present invention, for these unsaturated fatty acids to be positioned at the sn1, sn2 and/or sn3 position of the triglycerides which are, preferably, to be produced.

In a method or manufacturing process of the present invention the polynucleotides and polypeptides of the present invention may be used with at least one further polynucleotide encoding an enzyme of the fatty acid or lipid biosynthesis. Preferred enzymes are in this context the desaturases and elongases as mentioned above, but also polynucleotide encoding an enzyme having delta-8-desaturase and/or ddelta-9-elongase activity. All these enzymes reflect the individual steps according to which the end products of the method of the present invention, for example EPA or DHA are produced from the starting compounds linoleic acid (C18:2) or linolenic acid (C18:3). As a rule, these compounds are not generated as essentially pure products. Rather, small traces of the precursors may be also present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting host cell, organism, or the starting plant, the end products, such as EPA or DHA, are present as mixtures. The precursors should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, more preferably, not to more than 10% by weight, most preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only EPA or more preferably only DHA, bound or as free acids, is/are produced as end product(s) in the process of the invention in a host cell. If the compounds EPA and DHA are produced simultaneously, they are, preferably, produced in a ratio of at least 1:2 (DHA:EPA), more preferably, the ratios are at least 1:5 and, most preferably, 1:8. Fatty acid esters or fatty acid mixtures produced by the invention, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. DHA as a preferred long chain polyunsaturated fatty acid is present in the fatty acid esters or fatty acid mixtures in a concentration of, preferably, at least 0.1; 0.2; 0.3;

0.4; 0.5; 0.6; 0.7; 0.8; 0.9 or 1%, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the method of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9, 12-epoxyoctadeca-9, 11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12coctadecatrienoicacid), catalpic acid (9t11 t13c-octadecatrienoic acid), eleostearic acid (9c11 t13t-octadecatrienoic acid), jacaric acid (8c1 Ot12c-octadecatrienoic acid), punicic acid (9c11 t13c-octadecatrienoic acid), parinaric acid (9c11 t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c, 11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the process of the present invention, preferably, comprise less than 0.1 %, based on the total fatty acids, or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, C22:5M,8,12,15,21) and no nisinic acid (tetracosahexaenoic acid, C23:6L13,8,12,15,18,21).

Chemically pure VLC-PUFAs or fatty acid compositions can also be synthesized by the methods described herein. To this end, the fatty acids or the fatty acid compositions are isolated from a corresponding sample via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

The term "desaturase" encompasses all enzymatic activities and enzymes catalyzing the desaturation of fatty acids with different lengths and numbers of unsaturated carbon atom double bonds. Specifically this includes delta 4 (d4)-desaturase, catalyzing the dehydrogenation of the 4th and 5th carbon atom; Delta 5 (d5)-desaturase catalyzing the dehydrogenation of the 5th and 6th carbon atom; Delta 6 (d6)-desaturase catalyzing the dehydrogenation of the 6th and 7th carbon atom; Delta 8 (d8)-desaturase catalyzing the dehydrogenation of the 8th and 9th carbon atom; Delta 9 (d9)-desaturase catalyzing the dehydrogenation of the 9th and 10th carbon atom; Delta 12 (d12)-desaturase catalyzing the dehydrogenation of the 12th and 13th carbon atom; Delta 15 (d15)-desaturase catalyzing the dehydrogenation of the 15th and 16th carbon atom.

The terms "elongase" encompasses all enzymatic activities and enzymes catalyzing the elongation of fatty acids with different lengths and numbers of unsaturated carbon atom

double bonds. Especially the term "elongase" as used herein refers to the activity of an elongase, introducing two carbon molecules into the carbon chain of a fatty acid, preferably in the positions 5, 6, 9, 12 and/or 15 of fatty acids.

In the studies underlying this invention, enzymes with superior desaturase and elongase catalytic activities for the production of VLC-PUFA has been provided.

Polynucleotides encoding polypeptides which exhibit delta-6-elongase activity have been described in WO2001/059128, WO2004/087902 and WO2005/012316, said documents, describing this enzyme from Physcomitrella patens, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-5-desaturase activity have been described in WO2002026946 and WO2003/093482, said documents, describing this enzyme from Thraustochytrium sp., are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-6-desaturase activity have been described in WO2005/012316, WO2005/083093, WO2006/008099 and WO2006/069710, said documents, describing this enzyme from Ostreococcus tauri, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-6-elongase activity have been described in WO2005/012316, WO2005/007845 and WO2006/069710, said documents, describing this enzyme from Thalassiosira pseudonana, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-12-desaturase activity have been described for example in WO2006100241, said documents, describing this enzyme from Phytophthora sojae, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-4-desaturase activity have been described for example in WO2004/090123, said documents, describing this enzyme from Euglena gracilis, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-5-elongase activity have been described for example in WO2005/012316 and WO2007/096387, said documents, describing this enzyme from Ostreococcus tauri, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit omega 3-desaturase activity have been described for example in WO2008/022963, said documents, describing this enzyme from Phytium irregulare, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit omega 3-desaturase activity have been described for example in WO2005012316 and WO2005083053, said documents, describing this enzyme from Phytophthora infestans, are incorporated herein in their entirety.

Polynucleotides encoding polypeptides which exhibit delta-4-desaturase activity have been described for example in WO2002026946, said documents, describing this enzyme from Thraustochytrium sp., are incorporated herein in their entirety.

Polynucleotides coding for a delta-4 desaturase from Pavlova lutheri are described in WO2003078639 and WO2005007845. These documents are incorporated herein in their entirety, particularly in sofar as the documents relate to the delta-4 desaturase "PIDES 1"and figures 3a-3d of WO2003078639 and figures 3a, 3b of WO2005007845, respectively.

The polynucleotides encoding the aforementioned polypeptides are herein also referred to as "target genes".The polynucleotides are well known in the art. The sequences of said polynucleotides can be found in the sequence of the T-DNAs disclosed in the Examples section. E.g., the vector VC-LTM593-1qcz comprises the sequences of the said polynucleotides. The sequence of this vector is shown in SEQ ID NO: 3 (see table 193). The positions of the respective polynucleotides in this vector are indicted in table 11. E.g., the sequence of the delta-6 elongase from Physcomitrella patens comprises the sequence of nucleotides 1267 tp 2139 of SEQ ID NO: 3.

Polynucleotides encoding polypeptides which exhibit delta-15-desaturase activity have been described for example in WO2010/066703, said documents, describing this enzyme from Cochliobolus heterostrophus C5, are incorporated herein in their entirety. For the sequence of the polynucleotide encoding a polypeptides which exhibits delta-15-desaturase activity from Cochliobolus heterostrophus C5 see, e.g. nucleotides 2151 to 3654 of VC-LLM338-3qcz rc. The sequence of this vector is shown in SEQ ID NO: 9.

A polynucleotide encoding a polypeptide having a desaturase or elongase activity as specified above is obtainable or obtained in accordance with the present invention for example from an organism of genus Ostreococcus, Thraustochytrium, Euglena, Thalassiosira, Phytophthora, Phytium, Cochliobolus, Physcomitrella. However, orthologs, paralogs or other homologs may be identified from other species. Preferably, they are obtained from plants such as algae, for example Isochrysis, Mantoniella, Crypthecodinium, algae/diatoms such as Phaeodactylum, mosses such as Ceratodon, or higher plants such as the Primulaceae such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Entomophthora, Mucor or Mortierella, bacteria such as Shewanella, yeasts or animals. Preferred animals are nematodes such as Caenorhabditis, insects or vertebrates. Among

the vertebrates, the nucleic acid molecules may, preferably, be derived from Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus, more preferably, from the order of the Salmoniformes, most preferably, the family of the Salmonidae, such as the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario. Moreover, the nucleic acid molecules may be obtained from the diatoms such as the genera Thalassiosira or Phaeodactylum.

Thus, the term "polynucleotide" as used in accordance with the present invention further encompasses variants or derivatives of the aforementioned specific polynucleotides representing orthologs, paralogs or other homologs of the polynucleotide of the present invention. Moreover, variants or derivatives of the polynucleotide of the present invention also include artificially generated muteins. Said muteins include, e.g., enzymes which are generated by mutagenesis techniques and which exhibit improved or altered substrate specificity, or codon optimized polynucleotides.

Nucleic acid variants or derivatives according to the invention are polynucleotides which differ from a given reference polynucleotide by at least one nucleotide substitution, addition and/or deletion. If the reference polynucleotide codes for a protein, the function of this protein is conserved in the variant or derivative polynucleotide, such that a variant nucleic acid sequence shall still encode a polypeptide having a desaturase or elongase activity as specified above. Variants or derivatives also encompass polynucleotides comprising a nucleic acid sequence which is capable of hybridizing to the aforementioned specific nucleic acid sequences, preferably, under stringent hybridization conditions. These stringent conditions are known to the skilled in the art and can be found in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred example for stringent hybridization conditions are hybridization conditions in 6 ´ sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more wash steps in 0.2 ´ SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, with regard to the temperature and concentration of the buffer. For example, under "standard hybridization conditions" the temperature differs depending on the type of nucleic acid between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 ´ SSC (pH 7.2). If organic solvent is present in the abovementioned buffer, for example 50% formamide, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA: DNA hybrids are, preferably, 0.1 ´ SSC and 20°C to 45°C, preferably between 30°C and 45°C. The hybridization conditions for DNA:RNA hybrids are, preferably, 0.1 ´ SSC and 30°C to 55°C, preferably between 45°C and 55°C. The abovementioned hybridization temperatures are determined for example for a nucleic acid with approximately 100 bp (= base pairs) in length and a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the hybridization

conditions required by referring to textbooks such as the textbook mentioned above, or the following textbooks: Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford. Alternatively, polynucleotide variants are obtainable by PCR-based techniques such as mixed oligonucleotide primer based amplification of DNA, i.e. using degenerated primers against conserved domains of the polypeptides of the present invention. Conserved domains of the polypeptide of the present invention may be identified by a sequence comparison of the nucleic acid sequences of the polynucleotides or the amino acid sequences of the polypeptides of the present invention. Oligonucleotides suitable as PCR primers as well as suitable PCR conditions are described in the accompanying Examples. As a template, DNA or cDNA from bacteria, fungi, plants or animals may be used. Further, variants include polynucleotides comprising nucleic acid sequences which are at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or at least 99% identical to the nucleic acid coding sequences shown in any one of the T-DNA sequences given in the corresponding tables of the examples. Of course, the variants must retain the function of the respective enzyme, i.e. a variant of a delta-4-desaturase must retain delta-4-desaturase activity.

The percent identity values are, preferably, calculated over the entire amino acid or nucleic acid sequence region. A series of programs based on a variety of algorithms is available to the skilled worker for comparing different sequences. In a preferred embodiment, the percent identity between two amino acid sequences is determined using the Needleman and Wunsch algorithm (Needleman 1970, J. Mol. Biol. (48):444-453) which has been incorporated into the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), a BLOSUM62 scoring matrix, and a gap opening penalty of 10 and a gap entension pentalty of 0.5. Guides for local installation of the EMBOSS package as well as links to WEB-Services can be found at http://emboss.sourceforge.net. A preferred, non-limiting example of parameters to be used for aligning two amino acid sequences using the needle program are the default parameters, including the EBLOSUM62 scoring matrix, a gap opening penalty of 10 and a gap extension penalty of 0.5. In yet another preferred embodiment, the percent identity between two nucleotide sequences is determined using the needle program in the EMBOSS software package (EMBOSS: The European Molecular Biology Open Software Suite, Rice,P., Longden,I., and Bleasby,A, Trends in Genetics 16(6), 276-277, 2000), using the EDNAFULL scoring matrix and a gap opening penalty of 10 and a gap extension penalty of 0.5. A preferred, non-limiting example of parameters to be used in conjunction for aligning two nucleic acid sequences using the needle program are the default parameters, including the EDNAFULL scoring matrix, a gap opening penalty of 10 and a gap extension

penalty of 0.5. The nucleic acid and protein sequences of the present invention can further be used as a "query sequence" to perform a search against public databases to, for example, identify other family members or related sequences. Such searches can be performed using the BLAST series of programs (version 2.2) of Altschul et al. (Altschul 1990, J. Mol. Biol. 215:403-10). BLAST using desaturase and elongase nucleic acid sequences of the invention as query sequence can be performed with the BLASTn, BLASTx or the tBLASTx program using default parameters to obtain either nucleotide sequences (BLASTn, tBLASTx) or amino acid sequences (BLASTx) homologous to desaturase and elongase sequences of the invention. BLAST using desaturase and elongase protein sequences of the invention as query sequence can be performed with the BLASTp or the tBLASTn program using default parameters to obtain either amino acid sequences (BLASTp) or nucleic acid sequences (tBLASTn) homologous to desaturase and elongase sequences of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST using default parameters can be utilized as described in Altschul et al. (Altschul 1997, Nucleic Acids Res. 25(17):3389-3402).

A polynucleotide comprising a fragment of any nucleic acid, particularly of any of the aforementioned nucleic acid sequences, is also encompassed as a polynucleotide of the present invention. The fragments shall encode polypeptides which still have desaturase or elongase activity as specified above. Accordingly, the polypeptide may comprise or consist of the domains of the polypeptide of the present invention conferring the said biological activity. A fragment as meant herein, preferably, comprises at least 50, at least 100, at least 250 or at least 500 consecutive nucleotides of any one of the aforementioned nucleic acid sequences or encodes an amino acid sequence comprising at least 20, at least 30, at least 50, at least 80, at least 100 or at least 150 consecutive amino acids of any one of the aforementioned amino acid sequences.

The variant polynucleotides or fragments referred to above, preferably, encode polypeptides retaining desaturase or elongase activity to a significant extent, preferably, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80% or at least 90% of the desaturase or elongase activity exhibited by any of the polypeptide comprised in any of the T-DNAs given in the accompanying Examples.

Further enzymes beneficial for the present invention as described in detail in the examples are aclytransferases and transacylases (cf. WO 2011161093 A1). One group of acyltransferases having three distinct enzymatic activities are enzymes of the "Kennedy pathway", which are located on the cytoplasmic side of the membrane system of the endoplasmic reticulum (ER). The ER-bound acyltransferases in the microsomal fraction use acyl-CoA as the activated form of fatty acids. Glycerol-3-phosphate acyltransferase (GPAT) catalyzes the incorporation of acyl groups at the sn-1 position of glycerol-3-phosphate. 1-Acylglycerol-3-phosphate acyltransferase, also known as lysophosphatidic acid

acyltransferase (LPAAT), catalyze the incorporation of acyl groups at the sn-2 position of lysophosphatidic acid (LPA). After dephosphorylation of phosphatidic acid by phosphatidic acid phosphatase (PAP), diacylglycerol acyltransferase (DGAT) catalyzes the incorporation of acyl groups at the sn-3 position of diacylglycerols. Further enzymes directly involved in TAG biosynthesis - apart from the said Kennedy pathway enzymes - are the phospholipid diacylglycerol acyltransferase (PDAT), an enzyme that transfers acyl groups from the sn-2 position of membrane lipids to the sn-3 position of diacylglycerols, and diacylglyceroldiacylglycerol transacylase (DDAT), an enzyme that transfers acylgroups from the sn-2 position of one diacylglycerol-molecule to the sn-3 position of another diacylglycerol-molecule. Lysophospholipid acyltransferase (LPLAT) represents a class of acyltransferases that are capable of incorporating activated acyl groups from acyl-CoA to membrane lipids, and possibly catalyze also the reverse reaction. More specifically, LPLATs can have activity as lysophosphophatidylethanolamine acyltransferase (LPEAT) and lysophosphatidylcholine acyltransferase (LPCAT). Further enzymes, such as lecithin cholesterol acyltransferase (LCAT) can be involved in the transfer of acyl groups from membrane lipids into triacylglycerides, as well. The documents WO 98/54302 and WO 98/54303 disclose a human LPAAT and its potential use for the therapy of diseases, as a diagnostic, and a method for identifying modulators of the human LPAAT. Moreover, a variety of acyltransferases with a wide range of enzymatic functions have been described in the documents WO 98/55632, WO 98/55631 , WO 94/13814, WO 96/24674, WO 95/27791, WO 00/18889, WO 00/18889, WO 93/10241, Akermoun 2000, Biochemical Society Transactions 28: 713-715, Tumaney 1999, Biochimica et Biophysica Acta 1439: 47-56, Fraser 2000, Biochemical Society Transactions 28: 715- 7718, Stymne 1984, Biochem. J. 223: 305-314, Yamashita 2001, Journal of Biological Chemistry 276: 26745-26752, and WO 00/18889.

The term "expression control sequence" as used herein refers to a nucleic acid sequence which is capable of governing, i.e. initiating and controlling, transcription of a nucleic acid sequence of interest, in the present case the nucleic sequences recited above. Such a sequence usually comprises or consists of a promoter or a combination of a promoter and enhancer sequences. Expression of a polynucleotide comprises transcription of the nucleic acid molecule, preferably, into a translatable mRNA. Additional regulatory elements may include transcriptional as well as translational enhancers. The following promoters and expression control sequences may be, preferably, used in an expression vector according to the present invention. The cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoters are, preferably, used in Gram-negative bacteria. For Gram-positive bacteria, promoters amy and SPO2 may be used. From yeast or fungal promoters ADC1, AOX1r, GAL1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH are, preferably, used. For animal cell or organism expression, the promoters CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer are preferably used. From plants the promoters CaMV/35S (Franck 1980, Cell 21: 285-294], PRP1 (Ward 1993,

Plant. Mol. Biol. 22), SSU, OCS, lib4, usp, STLS1, B33, nos or the ubiquitin or phaseolin promoter. Also preferred in this context are inducible promoters, such as the promoters described in EP 0388186 A1 (i.e. a benzylsulfonamide-inducible promoter), Gatz 1992, Plant J. 2:397-404 (i.e. a tetracyclin-inducible promoter), EP 0335528 A1 (i.e. a abscisic-acid-inducible promoter) or WO 93/21334 (i.e. a ethanol- or cyclohexenol-inducible promoter). Further suitable plant promoters are the promoter of cytosolic FBPase or the ST-LSI promoter from potato (Stockhaus 1989, EMBO J. 8, 2445), the phosphoribosyl-pyrophosphate amidotransferase promoter from Glycine max (Genbank accession No. U87999) or the node-specific promoter described in EP 0249676 A1. Particularly preferred are promoters which enable the expression in tissues which are involved in the biosynthesis of fatty acids. Also particularly preferred are seed-specific promoters such as the USP promoter in accordance with the practice, but also other promoters such as the LeB4, DC3, phaseolin or napin promoters. Further especially preferred promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (napin promoter from oilseed rape), WO 98/45461 (oleosin promoter from Arobidopsis, US 5,504,200 (phaseolin promoter from Phaseolus vulgaris), WO 91/13980 (Bce4 promoter from Brassica), by Baeumlein et al., Plant J., 2, 2, 1992:233-239 (LeB4 promoter from a legume), these promoters being suitable for dicots. The following promoters are suitable for monocots: lpt-2 or lpt-1 promoter from barley (WO 95/15389 and WO 95/23230), hordein promoter from barley and other promoters which are suitable and which are described in WO 99/16890. In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. Likewise, it is possible and advantageous to use synthetic promoters, either additionally or alone, especially when they mediate a seed-specific expression, such as, for example, as described in WO 99/16890. In a particular embodiment, seed-specific promoters are utilized to enhance the production of the desired PUFA or VLC-PUFA.

The term "operatively linked" as used herein means that the expression control sequence and the nucleic acid of interest are linked so that the expression of the said nucleic acid of interest can be governed by the said expression control sequence, i.e. the expression control sequence shall be functionally linked to the said nucleic acid sequence to be expressed. Accordingly, the expression control sequence and, the nucleic acid sequence to be expressed may be physically linked to each other, e.g., by inserting the expression control sequence at the 5´end of the nucleic acid sequence to be expressed. Alternatively, the expression control sequence and the nucleic acid to be expressed may be merely in physical proximity so that the expression control sequence is capable of governing the expression of at least one nucleic acid sequence of interest. The expression control sequence and the nucleic acid to be expressed are, preferably, separated by not more than 500 bp, 300 bp, 100 bp, 80 bp, 60 bp, 40 bp, 20 bp, 10 bp or 5 bp.

Preferred polynucleotides of the present invention comprise, in addition to a promotor, a terminator sequence operatively linked to the nucleic acid sequence of interest.

The term "terminator" as used herein refers to a nucleic acid sequence which is capable of terminating transcription. These sequences will cause dissociation of the transcription machinery from the nucleic acid sequence to be transcribed. Preferably, the terminator shall be active in plants and, in particular, in plant seeds. Suitable terminators are known in the art and, preferably, include polyadenylation signals such as the SV40-poly-A site or the tk-poly-A site or one of the plant specific signals indicated in Loke et al. (Loke 2005, Plant Physiol 138, pp. 1457-1468), downstream of the nucleic acid sequence to be expressed.

The invention furthermore relates to recombinant nucleic acid molecules comprising at least one nucleic acid sequence which codes for a polypeptide having desaturase and/or elongase activity which is modified by comparison with the nucleic acid sequence in the organism from which the sequence originates in that it is adapted to the codon usage in one or more plant species.

For the purposes of the invention "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (=gene construct) or a vector comprising the nucleic acid sequences used in the process according to the invention or a host cell transformed with the nucleic acid sequences, expression cassette or vector used in the process according to the invention, all those constructions brought about by recombinant methods in which either the nucleic acid sequence, or a genetic control sequence which is operably linked with the nucleic acid sequence, for example a promoter, or are not located in their natural genetic environment or have been modified by recombinant methods.

Preferably, the plant cell of the present invention is an oilseed crop plant cell. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla. Preferred plants to be used for introducing the polynucleotide or T-DNA of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. It is to be understood that host cells derived from a plant may also be used for producing a plant according to the present invention. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Arecaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Compositae, Crypthecodiniaceae, Cruciferae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae,

Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae, Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae, Solanaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella,

Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, Elaeagnaceae such as the genus Elaeagnus, for example the genus and species Olea europaea [olive], Ericaceae such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], Medicago sativa, Medicago falcata, Medicago varia [alfalfa], Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max [soybean], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria con¬voluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygro¬metrica var. arctica, Funaria hygrometrica var.

calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physco¬mitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species Laurus nobilis [bay], Persea americana, Persea gratissima or Persea persea [avocado], Leguminosae, such as the genus Arachis, for example the genus and species Arachis hypogaea [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum [linseed], Lythrarieae, such as the genus Punica, for example the genus and species Punica granatum [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species Marchantia berteroana, Marchantia foliacea, Marchantia macropora, Musaceae, such as the genus Musa, for example the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species Oenothera biennis or Camissonia brevipes [evening primrose], Palmae, such as the genus Elacis, for example the genus and species Elaeis guineensis [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species Sesamum indicum [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel,

Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum [barley], Secale cereale [rye], Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida [oats], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower,

safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are sunflower, safflower, tobacco, mullein, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp, thistle or safflower. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed, or hemp, or. most preferred, plants of family Brassicaceae.

The invention is also concerned with providing constructs for establishing high content of VLC-PUFAs in plants or parts thereof, particularly in plant oils.

As such, the invention provides a T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and at least one expression cassette comprising a promotor, operatively linked thereto a target gene, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp. In an embodiment, the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

As described elsewhere herein, the the T-DNA of the present invention may comprise multiple expression cassettes encoding various, i.e. multiple proteins. In an embodiment, expression cassettes are separated from each other by a separator of at least 100 base pairs, preferably of 100-200 base pairs. Thus, there is a separator between each expression cassette.

The invention thus provides nucleic acids, i.e. polynucleotides. A polynucleotide according to the present invention is or comprises a T-DNA according to the present invention. Thus, a T-DNA according to the present invention is a polynucleotide, preferably a DNA, and most preferably a double stranded DNA. A "T-DNA" according to the invention is a nucleic acid capable of eventual integration into the genetic material (genome) of a plant. The skilled person understands that for such integration a transformation of respective plant material is required, prefered transformation methods and plant generation methods are described herein.

According to the invention are also provided nucleic acids comprising a T-DNA as defined according to the present invention. For example, a T-DNA of the present invention may be comprised in a circular nucleic acid, e.g. a plasmid, such that an additional nucleic acid section is present between the left and right border elements, i.e. "opposite" of the expression cassette(s) according to the present invention. Such circular nucleic acid may be mapped into a linear form using an arbitrary starting point, e.g. such that the definition "left border element – expression cassette – right border element – additional nucleic acid section opposite of the expression cassette" defines the same circular nucleic acid as the definition "expression cassette – right border element – additional nucleic acid section opposite of the expression cassette – left border element". The additional nucleic acid section preferably comprises one or more genetic elements for replication of the total nucleic acid, i.e. the nucleic acid molecule comprising the T-DNA and the additional nucleic acid section, in one or more host microorganisms, preferably in a microorganism of genus Escherichia, preferably E. coli, and/or Agrobacterium. Preferable host microorganisms are described below in more detail. Such circular nucleic acids comprising a T-DNA of the present invention are particularly useful as transformation vectors; such vectors and are described below in more detail.

The T-DNA length is preferably large, i.e. it has a minimum length of at least 30000 bp, preferably more than 30000 bp, more preferably at least 40000 bp, even more preferably at least 50000 bp and most preferably at least 60000 bp. Preferably, the length of the T-DNA is in a range of any of the aforementioned minimum lengths to 120000bp, more preferably in a range of any of the aforementioned minimum lengths to 100000bp, even more preferably in a range of any of the aforementioned minimum lengths to 90000 bp, even more preferably in a range of any of the aforementioned minimum lengths to 80000 bp. With such minimum lengths it is possible to introduce a number of genes in the form of expression cassettes such that each individual gene is operably liked to at least one promotor and at least one terminator. As is shown hereinafter in the examples section, the invention makes use of such minimum length T-DNA for introducing the genes required for the methabolic pathway of VLC-PUFA production in plants, e.g. oil seed plants, preferably plants of genus Brassica. Also, the length of the T-DNA is preferably limited as described before to allow for easy handling.

In 3' direction of the T-DNA left border element or in 5' direction of the T-DNA right border element, a separator is present setting the respective border element apart from the expression cassette comprising the target gene. The separator in 3' direction of the T-DNA left border element does not necessarily have the same length and/or sequence as the separator in 5' direction of the T-DNA right border element, as long as both separators suffice to the further requirements given below.

The separator or spacer is a section of DNA predominantly defined by its length. Its function is to separate a target gene from the T-DNA's left or right border, respectively. As will be shown in the examples, introducing a separator effectively separates the gene of interest from major influences exerted by the neighbouring genomic locations after insertion of the T-DNA into a genomic DNA. For example it is commonly believed that not all genomic loci are equally suitable for expression of a target gene, and that the same gene under the control of the same promotor and terminator may be expressed in different inensity in plants depending on the region of integration of the target gene (and its corresponding promotor and terminator) the plant genome. It is generally believed that different regions of a plant genome are accessible with differing ease for transcription factors and/or polymerase enzymes, for example due to these regions being tightly wound around histones and/or attached to the chromosomal backbone (cf. for example Deal et al., Curr Opin Plant Biol. Apr 2011; 14(2): 116–122) or other scaffold material (cf. e.g. Fukuda Y. , Plant Mol Biol. 1999 Mar; 39(5): 1051-62). The mechanism of achieving the above mentioned benefits by the T-DNA of the present invention is not understood it is convenient to think of the spacer as a means for physically providing a buffer to compensate for strain exerted by DNA winding by neighbouring histones or chromosomal backbone or other scaffold attached regions. As a model it can be thought that to transcribe a target gene, the DNA has to be partially unwound. If neighbouring regions of the target gene resist such unwinding, for example because they are tightly wound around histones or otherwise attached to a scaffold or backbone such that rotation of nucleic acid strands is limited, the spacer allows to distribute the strain created by the unwinding attempt over a longer stretch of nucleic acid, thereby reducing the force required for unwinding at the target gene.

In an embodiment, the separator has a length of at least 500 bp. The separator, thus, can be longer than 500 bp, and preferably is at least 800bp in length, more preferably at least 1000 bp. Longer spacers allow for even more physical separation between the target gene and the nearest genomic flanking region.

In another embodiment, the spacer has a length of at least 100 bp. Preferably, the spacer has a length of 100 to 200 base pairs.

The separator preferably has a sequence devoid of matrix or scaffold attachment signals. Preferably, the separator or spacer is does not comprise more than once for a length of 500 bp, preferably not more than once for a length of 1000 bp, a 5-tuple which occurs in the spacers described below in the examples for 20 or more times, summarized over all spacers given in the examples. Those tuples are, in increasing frequency in the spacers given in the examples: AGCCT, CGTAA, CTAAC, CTAGG, GTGAC, TAGGC, TAGGT, AAAAA, AACGC, TTAGC, ACGCT, GCTGA, ACGTT, AGGCT, CGTAG, CTACG, GACGT, GCTTA, AGCTT, CGCTA, TGACG, ACGTG, AGCTG, CACGT, CGTGA, CGTTA, AGCGT, TCACG, CAGCT, CGTCA, CTAGC, GCGTC, TTACG, GTAGC, TAGCG, TCAGC, TAGCT,

AGCTA, GCTAG, ACGTA, TACGT. By reducing the frequency of occurrence of one or more of the aforelisted tuples compared to the separators or spacers as given in the examples, a further increase in expression of a target gene in the T-DNA can be achieved.

The separator may contain a selectable marker. A selectable marker is a nucleic acid section whose presence preferably can be verified in seed without having to wait for the sprouting or full growth of the plant. Preferably the selectable marker conveys a phenotypical property to seed or to a growing plant, for example herbicide tolerance, coloration, seed surface properties (e.g. wrinkling), luminescence or fluorescence proteins, for example green fluorecent protein or luciferase. If for exhibiting the phenotypical feature an expression of a marker gene is required, then the separator correspondingly comprises the marker gene as selectable marker, preferably in the form of an expression cassette. Inclusion of a selectable marker in the separator is particularly advantageous since the marker allows to easily discard non-transformant plant material. Also, in such unexpected case where the T-DNA integrates in a location of the plant genome where the length and/or nucleobase composition of the spacer is insufficient to overcome gene silencing effects caused by the neighbouring genomic DNA, the selectable marker allows to easily discard such unfortunately badly performing exceptional transformants. Thus, preferably the separator comprises an expression cassette for expression of a herbicide tolerance gene. Such separator greatly reduces the chance of having to cultivate a transformant where silencing effects are so strong that even the expression of the selectable marker gene is greatly reduced or fully inhibited. According to the invention, the separator preferably does not comprise a desaturase or elongase gene, and also preferably does not comprise a promotor or operatively linked to a desaturase or elongase gene. Thus, the T-DNA of the present invention in preferred embodiments is useful for effective separation of the desaturase and elongase genes essential for the production of VLC-PUFAs from any influence of effects caused by neighbouring genomic plant DNA.

For the production of VLC-PUFAs in plants, the invention also provides a T-DNA comprising the coding sequences of any single of the tables given in the examples, preferably comprising the coding sequences and and promotors of any single of the tables given in the examples, more preferably the coding sequences and promotors and terminators of any single of the tables given in the examples, and most preferably the expression cassettes of any single of the tables of the examples.

Also, the invention provides a T-DNA for production of VLC-PUFAs in a plant, wherein the T-DNA comprises a left and a right border element and in between one or more expression cassette(s), wherein the length of the T-DNA, measured from left to right border element and comprising the one ore more expression cassettes, has a length of at least 30000 bp. In an embodiment, the expression cassette(s) closest to a left or right border element, respectively, is (are) separated from said closest border element of the T-DNA by a

separator of at least 500 bp length. It is to be understood that the one or more expression cassette(s) each comprise a promotor, operatively linked to a target gene, and downstream thereof a terminator, wherein the target gene of a respective expression cassette is a desaturase or elongase gene as required for production of a VLC-PUFA. Preferably at least one and most preferably all of the target genes codes/code for a desaturase or elongase as given in any of the tables in the examples section, and further preferably at least one and most preferably all expression cassettes consist of a combination of promotor, desaturase/elongase gene and terminator as given in any of the examples below.

In one embodiment a plant of the invention comprises one or more T-DNA of the present invention comprising one or more expression cassetts encoding for one or more d6Des, one or more d6Elo, one or more d5Des, one or more o3Des, one or more d5Elo and one or more D4Des, preferably for at least one CoA-dependent D4Des and one Phospholipid-dependent d4Des. In one embodiment, the T-DNA encodes also one or more d12Des.

In one embodiment, the plant of the invention or a part thereof as described herein comprises one or more T-DNAs of the invention which encode for at least two d6Des, at least two d6Elo and/or, at least two o3Des. In one embodiment, the present plant of the invention or a part thereof comprise a T-DNA comprising one or more expression cassets encoding for at least one CoA-depenent d4Des and at least one phopho-lipid dependent d4Des. In one embodiment the activities of the enzymes expressed by the one or more T-DNAs in the plant of the invention or a prat thereof are encode and express polpeptides having the activities shown in column 1 of Table 19. In one embodiment the plant of the invention comprises at least one T-DNA as shown in columns 1 to 9 in Table 13. In one embodiment, one T-DNA of the present invention comprises one or more gene expression cassetts encoding for the activities or enzymes listed in Table 13 flanked by the R-regions and one flanked by the R2 regions.

In one embodiment, the at least one T-DNA further comprises an expression cassette which encodes for at least one d12Des. In one embodiemt, the T-DNA or T-DNAs comprise one or more expression cassettes encoding one or more d5Des(Tc_GA), o3Des(Pir_GA), d6Elo(Tp_GA) and/or d6Elo(Pp_GA). Such plants of the present invention have shown particularly high amounts and concentrations of VLC-PUFAs over three or more generations and under different growth conditions

In one embodiment the T-DNA of the invention encodes for the activites of column 1 as disclosed in Table 19, preferably of gene combinations disclosed in Table 13, even more preferred for promoter-gene combinations as described in Table 13

As described in detail in the examples, with regards to the production of VLC-PUFAs three desaturase genes are particularly prone to gene dosage effects (also called "copy number effects"), such that increasing the number of expression cassettes comprising these respective genes leads to a stronger increase in VLC-PUFA levels in plant oils than

increasing the number of expression cassettes of other genes. These genes are the genes coding for delta-12-desaturase activity, for delta-6-desaturase activity and omega-3-desaturase activity. Thus, according to the invention each expression cassette comprising a gene coding for a delta-12-desaturase, delta-6-desaturase or omega-3-desaturase is separated from the respective closest left or right border element by a separator and optionally one or more expression cassettes. It is to be understood that where the T-DNA of the present invention comprises more than one expression cassette comprising a gene of the same function, these genes do not need to be identical concerning their nucleic acid sequence or the polypeptide sequence encoded thereby, but should be functional homologs. Thus, for example, to make use of the gene dosage effect described herein a T-DNA according to the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-6-desaturases and/or omega-3-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a delta-12-desaturase, wherein the delta-12-desaturase polypeptides coded by the respective genes differ in their amino acid sequence. Likewise, a T-DNA of the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-12-desaturases and/or omega-3-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a delta-6-desaturase, wherein the delta-6-desaturase polypeptides coded by the respective genes differ in their amino acid sequence, or a T-DNA of the present invention may comprise, in addition to optionally a multiplicity of genes coding for delta-12-desaturases and/or delta-6-desaturases, two, three, four or more expression cassettes each comprising a gene coding for a omega-3-desaturase, wherein the omega-3-desaturase polypeptides coded by the respective genes differ in their amino acid sequence. According to the invention, the T-DNA may also comprise, instead of one or more of the afore mentioned coding sequences, a functional homolog thereof. A functional homolog of a coding sequence is a sequence coding for a polypeptide having the same metabolic function as the replaced coding sequence. For example, a functional homolog of a delta-5-desaturase would be another delta-5-desaturase, and a functional homolog of a delta-5-elongase would be another delta-5-elongase. The functional homolog of a coding sequence preferably codes for a polypeptide having at least 40% sequence identity to the polypeptide coded for by the corresponding coding sequence given in the corresponding table of the examples, more preferably at least 41%, more preferably at least 46%, more preferably at least 48%, more preferably at least 56%, more preferably at least 58%, more preferably at least 59%, more preferably at least 62%, more preferably at least 66%, more preferably at least 69%, more preferably at least 73%, more preferably at least 75%, more preferably at least 77%, more preferably at least 81%, more preferably at least 84%, more preferably at least 87%, more preferably at least 90%, more preferably at least 92%, more preferably at least 95%, more preferably at least 96%, more preferably at least 97%, more preferably at least 98% and even more preferably at least 99%. Likewise, a functional homolog of a promotor is a sequence for starting transcription of a coding sequence located within 500 bp for a proximal promotor or, for a distal promotor, within 3000 bp distant from the promotor

TATA box closest to the coding sequence. Again, a functional homolog of a plant seed specific promotor is another plant seed specific promotor. The functional homolog of a terminator, correspondingly, is a sequence for ending transcription of a nucleic acid sequence.

The examples describe particularly preferred T-DNA sequences. As described above, the skilled person understands that the coding sequences, promotors and terminators described therein can be replaced by their functional homologs. However, the examples also describe that according to the invention, certain combinations of promotors and coding sequences, or certain combinations of promotors driving the expression of their corresponding coding sequences, or certain coding sequences or combinations thereof are particularly advantageous; such combinations or individual coding sequences should according to the invention not be replaced by functional homologs of the respective element (here: coding sequence or promotor).

A T-DNA of the present invention preferably comprises two or more genes, preferably all genes, susceptible to a gene dosage effect. As described herein, it is advantageous for achieving high conversion efficiencies of certain enzymatic acitvities, e.g. delta-12-desaturase, delta-6-desaturase and/or omega-3-desaturase activity, to introduce more than one gene coding for an enzyme having the desired activity into a plant cell. When introducing T-DNA into plant cells, generally transformation methods involving exposition of plant cells to microorganisms are employed, e.g. as described herein. As each microorganism may comprise more than one nucleic acid comprising a T-DNA of the present invention, recombinant plant cells are frequently obtained comprising two or more T-DNAs of the present invention independently integrated into the cell's genetic material. Thus, by combining genes susceptible to a gene dosage effect on one construct for transformation allows to easily exploit the independence of transformations to achieve a higher frequency of multiple insertions of such T-DNAs. This is particularly useful for transformation methods relying on co-transformation to keep the size of each construct to be transformed low. Also, where a construct comprises all genes susceptible to a gene dosage effect it is beneficially easy to increase the the number of such genes in a plant cell by over-transformation, e.g. transforming a plant cell with the T-DNA of the present invention, wherein the plant cell had previously been transformed with the same T-DNA or other genetic material.

The invention accordingly also provides a construct comprising a T-DNA according to the present invention, wherein the construct preferably is a vector for transformation of a plant cell by microorganism-mediated transformation, preferably by Agrobacterium-mediated transformation. Correspondingly, the invention also provides a transforming microorganism comprising one T-DNA according to the present invention, preferably as a construct comprising said T-DNA. Preferably the microorganism is of genus Agrobacterium,

preferably a disarmed strain thereof, and preferably of species Agrobacterium tumefaciens or, even more preferably, of species Agrobacterium rhizogenes. Corresponding strains are for example described in WO06024509A2, and methods for plant transformation using such microorganisms are for example described in WO13014585A1. These WO publications are incorporated herein in their entirety, because they contain valuable information about the creation, selection and use of such microorganisms.

The term "vector", preferably, encompasses phage, plasmid, viral vectors as well as artificial chromosomes, such as bacterial or yeast artificial chromosomes. Moreover, the term also relates to targeting constructs which allow for random or site-directed integration of the targeting construct into genomic DNA. Such target constructs, preferably, comprise DNA of sufficient length for either homolgous or heterologous recombination as described in detail below. The vector encompassing the polynucleotide of the present invention, preferably, further comprises selectable markers for propagation and/or selection in a host. The vector may be incorporated into a host cell by various techniques well known in the art. If introduced into a host cell, the vector may reside in the cytoplasm or may be incorporated into the genome. In the latter case, it is to be understood that the vector may further comprise nucleic acid sequences which allow for homologous recombination or heterologous insertion. Vectors can be introduced into prokaryotic or eukaryotic cells via conventional transformation or transfection techniques. The terms "transformation" and "transfection", conjugation and transduction, as used in the present context, are intended to comprise a multiplicity of prior-art processes for introducing foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate, rubidium chloride or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, carbon-based clusters, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory manuals, such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey. Alternatively, a plasmid vector may be introduced by heat shock or electroporation techniques. Should the vector be a virus, it may be packaged in vitro using an appropriate packaging cell line prior to application to host cells.

Preferably, the vector referred to herein is suitable as a cloning vector, i.e. replicable in microbial systems. Such vectors ensure efficient cloning in bacteria and, preferably, yeasts or fungi and make possible the stable transformation of plants. Those which must be mentioned are, in particular, various binary and co-integrated vector systems which are suitable for the T DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they contain at least the vir genes, which are required for the Agrobacterium-mediated transformation, and the sequences which delimit the T-DNA (T-

DNA border). These vector systems, preferably, also comprise further cis-regulatory regions such as promoters and terminators and/or selection markers with which suitable transformed host cells or organisms can be identified. While co-integrated vector systems have vir genes and T-DNA sequences arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. As a consequence, the last-mentioned vectors are relatively small, easy to manipulate and can be replicated both in E. coli and in Agrobacterium. These binary vectors include vectors from the pBIB-HYG, pPZP, pBecks, pGreen series. Preferably used in accordance with the invention are Bin19, pBI101, pBinAR, pGPTV and pCAMBIA. An overview of binary vectors and their use can be found in Hellens et al, Trends in Plant Science (2000) 5, 446–451. Furthermore, by using appropriate cloning vectors, the polynucleotides can be introduced into host cells or organisms such as plants or animals and, thus, be used in the transformation of plants, such as those which are published, and cited, in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42, 205-225.

More preferably, the vector of the present invention is an expression vector. In such an expression vector, i.e. a vector which comprises the polynucleotide of the invention having the nucleic acid sequence operatively linked to an expression control sequence (also called "expression cassette") allowing expression in prokaryotic plant cells or isolated fractions thereof.

Most important, the invention also provides a plant or seed thereof, comprising, integrated in its genome, a T-DNA of the present invention.

Such T-DNA preferably allows for the expression of all genes required for production of VLC-PUFAs in plants and particularly in the sees thereof, particularly in oilseed plants, and most beneficially in plants or seeds of family Brassicaceae, preferably of genus Brassica and most preferably of a species comprising a genome of one or two members of the species Brassica oleracea, Brassica nigra and Brassica rapa, thus preferably of the species Brassica napus, Brassica carinata, Brassica juncea, Brassica oleracea, Brassica nigra or Brassica rapa. Particularly preferred according to the invention are plants and seeds of the species Brassica napus and Brassica carinata.

The plants of the present invention are necessarily transgenic, i.e. they comprise genetic material not present in corresponding wild type plant or arranged differently in

corresponding wild type plant, for example differring in the number of genetic elements. For example, the plants of the present invention comprise promotors also found in wild type plants, but the plants of the present invention comprise such promotor operatively linked to a coding sequence sucht that this combination of promotor and coding sequence is not found in the corresponding wild type plant.

The plants and seeds of the present invention differ from hitherto produced plants for production of VLC-PUFAS in a number of advantageous features, some of which are described in detail in the examples. In particular, T-DNAs of the present invention allow for the generation of transformant plants (also called "recombinant plants" and seeds thereof with a high transformation frequency, with a high stability of T-DNA insertions over multiple generations of self-fertilized plants, unchanged or unimpaired phenotypical and agronomic characteristics other than VLC-PUFA production, and with high amounts and concentration of VLC-PUFAs, particularly EPA and/or DHA, in the oil of populations of such transformed plants and their corresponding progeny. The latter feature is particularly striking considering that even one full integration of the T-DNA required for establishing the metabolic pathway for VLC-PUFA production, optionally with an additional integration of a partial T-DNA of the present invention, is enough for reliable production of VLC-PUFAs and particularly of EPA and/or DHA in the combined seeds of corresponding populations of plant clones.

While previously reported expression of VLC-PUFAs either focus on the PUFA concentrations or amounts found in individual seeds, such reported amounts or concentrations are of limited value for deciding on agronomical usefulness, because seed-to-seed variability and plant-to-plant variability in VLC-PUFA amounts per seed are high even for identical clones cultivated side by side under identical greenhouse conditions. Also, it has now been found and is reported below in the examples that the contentration of VLC-PUFAs is negatively correlated with seed oil content in Brassica napus, such that the mere statement of VLC-PUFA concentration in %(w/w) of all fatty acids of a plant oil is not indicative of the VLC-PUFA amount achievable by agricultural (i.e. large scale) growing of corresponding clones. In summary, it has been notoriously difficult to distil from reported VLC-PUFA amounts or concentrations which genes, promotors, gene-promotor combinations, gene-gene combinations etc. are beneficial for VLC-PUFA synthesis in oilseed plants.

Also, it is important to note that VLC-PUFA amounts or concentrations can indeed be increased by increasing the number of expression cassettes of functionally identical genes. And it is also important to note that even though many prior art documents purport to comprise applicable technical teachings, e.g. to combine desaturases having a particular property, e.g. CoA-dependence, it is not known how to reduce such alleged technical teachings to practice, particularly as such documents only teach requirements (i.e. functional claim features) but not solutions for these requirements (i.e. structural features).

For example, such prior art documents frequently comprise only one example of a single gene and leave the reader with the instruction to start a research program to find other enzymes satisfying the functional definition given therein, provided that any other enzyme exists that satisfies those functional requirements.

Unless stated otherwise, a plant of the present invention comprising a T-DNA of the present invention can also be a plant comprising a part of a T-DNA of the present invention, where such part is sufficient for the production of a desaturase and/or elongase coded for in the corresponding full T-DNA of the present invention. Such plants most preferably comprise at least one full T-DNA of the present invention in addition to the part of a T-DNA of the present invention as defined in the previous sentence. Such plants are hereinafter also termed "partial double copy" plants. Event LBFDAU is an example of a plant comprising a part of a T-DNA of the present invention, and still being a plant of the present invention. In one embodiment the T_DNA is a full T-DNA.

Preferred plants of the present invention comprise one or more T-DNA(s) of the present invention comprising expression cassettes comprising, one or more genes encoding for one or more d5Des, one or more d6Elo, one or more d6Des, one or more o3Des, one or more d5Elo and one or more D4Des, preferably for at least one CoA-dependent D4Des and one Phospholipid-dependent d4Des. In one embodiment, at least one T-DNA further comprises a expression cassette which encodes for at least one d12Des. In one embodiemt, the T-DNA or T-DNAs further comprise one or more expression cassettes encoding one or more d5Des(Tc_GA), o3Des(Pir_GA), d6Elo(Tp_GA) and/or d6Elo(Pp_GA). Such plants of the present invention have shown particularly high amounts and concentrations of VLC-PUFAs over three or more generations and under different growth conditions.

Preferably, the plant cell of the present invention is an oilseed crop plant cell. More preferably, said oilseed crop is selected from the group consisting of flax (Linum sp.), rapeseed (Brassica sp.), soybean (Glycine and Soja sp.), sunflower (Helianthus sp.), cotton (Gossypium sp.), corn (Zea mays), olive (Olea sp.), safflower (Carthamus sp.), cocoa (Theobroma cacoa), peanut (Arachis sp.), hemp, camelina, crambe, oil palm, coconuts, groundnuts, sesame seed, castor bean, lesquerella, tallow tree, sheanuts, tungnuts, kapok fruit, poppy seed, jojoba seeds and perilla. Preferred plants to be used for introducing the polynucleotide or T-DNA of the invention are plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Preferred plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Arecaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Compositae, Crypthecodiniaceae, Cruciferae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Malvaceae, Moringaceae, Marchantiaceae, Onagraceae,

Olacaceae, Oleaceae, Papaveraceae, Piperaceae, Pedaliaceae, Poaceae, Solanaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes. Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, such as the genus and species Physcomitrella patens, Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species Pistacia vera [pistachio], Mangifer indica [mango] or Anacardium occidentale [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], Centaurea cyanus [cornflower], Cichorium intybus [chicory], Cynara scolymus [artichoke], Helianthus annus [sunflower], Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana, Locusta communis, Valeriana locusta [salad vegetables], Tagetes lucida, Tagetes erecta or Tagetes tenuifolia [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species Daucus carota [carrot], Betulaceae, such as the genus Corylus, for example the genera and species Corylus avellana or Corylus colurna [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species Borago officinalis [borage], Brassicaceae, such as the genera Brassica, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species Brassica napus, Brassica rapa ssp. [oilseed rape], Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides, Melanosinapis communis [mustard], Brassica oleracea [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species Anana comosus, Ananas ananas or Bromelia comosa [pineapple], Caricaceae, such as the genus Carica, such as the genus and species Carica papaya [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species Cannabis sativa [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba or Convolvulus panduratus [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species Beta vulgaris, Beta vulgaris var. altissima, Beta vulgaris var.Vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva or Beta vulgaris var. esculenta [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for example the genus and species Cryptecodinium cohnii, Cucurbitaceae, such as the genus Cucurbita, for example the genera and species Cucurbita maxima, Cucurbita mixta, Cucurbita pepo or Cucurbita moschata [pumpkin/squash], Cymbellaceae such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species Phaeodactylum tricornutum, Ditrichaceae such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania,

Trichodon, Skottsbergia, for example the genera and species Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpureus, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon, purpureus spp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus, Elaeagnaceae such as the genus Elaeagnus, for example the genus and species Olea europaea [olive], Ericaceae such as the genus Kalmia, for example the genera and species Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros or Kalmia lucida [mountain laurel], Euphorbiaceae such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta [manihot] or Ricinus communis [castor-oil plant], Fabaceae such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, Soja, for example the genera and species Pisum sativum, Pisum arvense, Pisum humile [pea], Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbek, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa [silk tree], Medicago sativa, Medicago falcata, Medicago varia [alfalfa], Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida or Soja max [soybean], Funariaceae such as the genera Aphanorrhegma, Entosthodon, Funaria, Physcomitrella, Physcomitrium, for example the genera and species Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria con¬voluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygro¬metrica var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis,

Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physco¬mitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense, Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species Cocos nucifera, Pelargonium grossularioides or Oleum cocois [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra or Wallia nigra [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species Laurus nobilis [bay], Persea americana, Persea gratissima or Persea persea [avocado], Leguminosae, such as the genus Arachis, for example the genus and species Arachis hypogaea [peanut], Linaceae, such as the genera Linum, Adenolinum, for example the genera and species Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense or Linum trigynum [linseed], Lythrarieae, such as the genus Punica, for example the genus and species Punica granatum [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum or Gossypium thurberi [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species Marchantia berteroana, Marchantia foliacea, Marchantia macropora, Musaceae, such as the genus Musa, for example the genera and species Musa nana, Musa acuminata, Musa paradisiaca, Musa spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species Oenothera biennis or Camissonia brevipes [evening primrose], Palmae, such as the genus Elacis, for example the genus and species Elaeis guineensis [oil palm], Papaveraceae, such as the genus Papaver, for example the genera and species Papaver orientale, Papaver rhoeas, Papaver dubium [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species Sesamum indicum [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe

elongata, Peperomia elongata, Piper elongatum, Steffensia elongata [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon, Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum [barley], Secale cereale [rye], Avena sativa, Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida [oats], Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia [rice], Zea mays [maize], Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum or Triticum vulgare [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species Porphyridium cruentum, Proteaceae, such as the genus Macadamia, for example the genus and species Macadamia intergrifolia [macadamia], Prasinophyceae such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica, Mantoniella squamata, Ostreococcus tauri, Rubiaceae such as the genus Cofea, for example the genera and species Cofea spp., Coffea arabica, Coffea canephora or Coffea liberica [coffee], Scrophulariaceae such as the genus Verbascum, for example the genera and species Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum or Verbascum thapsus [mullein], Solanaceae such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens [pepper], Capsicum annuum [paprika], Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris [tobacco], Solanum tuberosum [potato], Solanum melongena [eggplant], Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium or Solanum lycopersicum [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species Theobroma cacao [cacao] or Theaceae, such as the genus Camellia, for example the genus and species Camellia sinensis [tea]. In particular preferred plants to be used as transgenic plants in accordance with the present invention are oil fruit crops which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica,

evening primrose, mullein, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut, walnut) or crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oilseed crop plants.

Most preferably, the plant of the present invention is a plant found in the "Triangle of U", i.e. a plant of genus Brassica: Brassica napus (AA CC genome; n=19) is an amphidiploid plant of the Brassica genus but is thought to have resulted from hybridization of Brassica rapa (AA genome; n=10) and Brassica oleracea (CC genome; n=9). Brassica juncea (AA BB genome; n=18) is an amphidiploid plant of the Brassica genus that is generally thought to have resulted from the hybridization of Brassica rapa and Brassica nigra (BB genome; n=8). Under some growing conditions, B. juncea may have certain superior traits to B. napus. These superior traits may include higher yield, better drought and heat tolerance and better disease resistance. Brassica carinata (BB CC genome; n=17) is an amphidiploid plant of the Brassica genus but is thought to have resulted from hybridization of Brassica nigra and Brassica oleracea. Under some growing conditions, B. carinata may have superior traits to B. napus. Particularly, B. carinata allows for an increase in VLC-PUFA concentrations by at least 20% compared to B. napus when transformed with the same T-DNA.

The plant of the present invention preferably is a "Canola" plant. Canola is a genetic variation of rapeseed developed by Canadian plant breeders specifically for its oil and meal attributes, particularly its low level of saturated fat. Canola herein generally refers to plants of Brassica species that have less than 2% erucic acid (Delta 13-22:1) by weight in seed oil and less than 30 micromoles of glucosinolates per gram of oil free meal. Typically, canola oil may include saturated fatty acids known as palmitic acid and stearic acid, a monounsaturated fatty acid known as oleic acid, and polyunsaturated fatty acids known as linoleic acid and linolenic acid. Canola oil may contain less than about 7%(w/w) total saturated fatty acids (mostly palmitic acid and stearic acid) and greater than 40%(w/w) oleic acid (as percentages of total fatty acids). Traditionally, canola crops include varieties of Brassica napus and Brassica rapa. Preferred plants of the present invention are spring canola (Brassica napus subsp. oleifera var. annua) and winter canola (Brassica napus subsp. oleifera var. biennis). Furthermore a canola quality Brassica juncea variety, which has oil and meal qualities similar to other canola types, has been added to the canola crop family (U.S. Pat. No. 6,303,849, to Potts et al., issued on Oct. 16, 2001; U.S. Pat. No. 7,423,198, to Yao et al.; Potts and Males, 1999; all of which are incorporated herein by reference). Likewise it is possible to establish canola quality B. carinata varieties by crossing canola quality variants of Brassica napus with Brassica nigra and appropriately selecting progeny thereof, optionally after further back-crossing with B. carinata, B. napus and/or B. nigra.

The invention also provides a plant or seed thereof of family Brassicaceae, preferably of genus Brassica, with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

i) crossing a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant comprising a T-DNA of the present invention and/or part of such T-DNA, with a parent plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant not comprising said T-DNA and/or part thereof, to yield a F1 hybrid,

ii) selfing the F1 hybrid for at least one generation, and

iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 12% (w/w) and/or the content of DHA is at least 2% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w).

This method allows to effectively incorporate genetic material of other members of family Brassicaceae, preferably of genus Brassica, into the genome of a plant comprising a T-DNA of the present invention. The method is particularly useful for combining a T-DNA of the present invention with genetic material responsible for beneficial traits exhibited in other members of family Brassicaceae. Beneficial traits of other members of family Brassicaceae are exemplarily described herein, other beneficial traits or genes and/or regulatory elements involved in the manifestation of a beneficial trait may be described elsewhere.

The parent plant not comprising the T-DNA of the present invention or part thereof preferably is an agronomically elite parent. In particular, the present invention teaches to transfer heterologous material from a plant or seed of the present invention to a different genomic background, for example a different variety or species.

In particular, the invention teaches to transfer the T-DNA or part thereof (the latter is particularly relevant for those plants of the present invention which comprise, in addition to a full T-DNA of the present invention, also a part of a T-DNA of the present invention, said part preferably comprising at least one expression cassette, the expression cassette preferably comprising a gene coding for a desaturase or elongase, preferably a delta-12-desaturase, delta-6-desaturase and/or omega-3-desaturase) into a species of genus Brassica carinata, or to introduce genetic material from Brassica carinata or Brassica nigra into the plants of the present invention comprising the T-DNA of the present invention and/or a part or two or more parts thereof. According to the invention, genes of Brassica

nigra replacing their homolog found in Brassica napus or added in addition to the homolog found in Brassica napus are particularly helpful in further increasing the amount of VLC-PUFAs in plant seeds and oils thereof.

Also, the invention teaches novel plant varieties comprising the T-DNA and/or part thereof of the present invention. Such varieties can, by selecting appropriate mating partners, be particularly adapted e.g. to selected climatic growth conditions, herbicide tolerance, stress resistance, fungal resistance, herbivore resistance, increased or reduced oil content or other beneficial features. As shown hereinafter in the examples it is particularly beneficial to provide plants of the present invention wherein the oil content thereof at harvest is lower than that of corresponding wild type plants of the same variety, such as to improve VLC-PUFA amounts in the oil of said plants of the present invention and/or VLC-PUFA concentrations in said oil.

Also, the invention provides a method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

i) crossing a transgenic plant of the invention with a parent plant not comprising a T-DNA of the present invention or part thereof, said parent plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a F1 hybrid,

ii) selfing the F1 hybrid for at least one generation, and

iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 8% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), preferably at an oil content of 35% (w/w), and more preferably at an oil content of 40% (w/w).

The method allows the creation of novel variants and transgenic species of plants of the present invention, and the seeds thereof. Such plants and seeds exhibit the aforementioned benefits of the present invention. Preferably, the content of EPA is at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content of the oil. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the oil. The present invention for the first time allows to achieve such high levels of VLC-PUFA in seed reliably under agronomic conditions, i.e. representative for the real yield obtained from seeds of a commercial field of at least 1 ha planted with plants of the present invention, wherein the plants have a defined copy number of genes for implementing the pathway for production of EPA and/or DHA in said plants, and the copy number being low, i.e. single-copy or partial double copy.

A plant of the present invention also includes plants obtainable or obtained by backcrossing (cross into the non-transgenic, isogenic parent line), and by crossing with other germplasms of the Triangle of U. Accordingly, the invention provides a method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from a progeny line prepared by a method comprising the steps of

i) crossing a transgenic plant of the invention (also called "non-recurring parent") with a parent plant not expressing a gene comprised in the T-DNA of the present invention, said parent plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a hybrid progeny,

ii) crossing the hybrid progeny again with the parent to obtain another hybrid progeny,

iii) optionally repeating step ii) and

iv) selecting a hybrid progeny comprising the T-DNA of the present invention.

Backcrossing methods, e.g. as described above, can be used with the present invention to improve or introduce a characteristic into the plant line comprising the T-DNA of the present invention. Such hybrid progeny is selected in step iv) which suffices predetermined parameters. The backcrossing method of the present invention thereby beneficially facilitates a modification of the genetic material of the recurrent parent with the desired gene, or preferably the T-DNA of the present invention, from the non-recurrent parent, while retaining essentially all of the rest of the desired genetic material of the recurrent parent, and therefore the desired physiological and morphological, constitution of the parent line. The selected hybrid progeny is then preferably multiplied and constitutes a line as described herein. Selection of useful progeny for repetition of step ii) can be further facilitated by the use of genomic markers. For example, such progeny is selected for the repetition of step ii) which comprises, compared to other progeny obtained in the previous crossing step, most markers also found in the parent and/or least markers also found in the non-recurring parent except the desired T-DNA of the present invention or part thereof.

Preferably, a hybrid progeny is selected which comprises the T-DNA of the present invention, and even more preferably also comprises at least one further expression cassette from the non-recurring parent of the present invention, e.g. by incorporation of an additional part of the T-DNA of the present invention into the hybrid plant genetic material.

Further preferably a hybrid progeny is obtained wherein essentially all of the desired morphological and physiological characteristics of the parent are recovered in the converted plant, in addition to genetic material from the non-recurrent parent as determined at the 5% significance level when grown under the same environmental conditions.

Further preferably, a hybrid progeny is selected which produces seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 8% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), preferably at an oil content of 35% (w/w), and more preferably at an oil content of 40% (w/w).

It is to be understood that such seed VLC-PUFA content is to be measured not from a single seed or from the seeds of an individual plant, but refer to the numeric average of seed VLC-PUFA content of at least 100 plants, even more preferably of at least 200 plants, even more preferably of at least 200 plants half of which have been grown in field trials in different years.

The choice of the particular non-recurrent parent will depend on the purpose of the backcross. One of the major purposes is to add some commercially desirable, agronomically important trait to the line.

The term "line" refers to a group of plants that displays very little overall variation among individuals sharing that designation. A "line" generally refers to a group of plants that display little or no genetic variation between individuals for at least one trait. A "DH (doubled haploid) line," as used in this application refers to a group of plants generated by culturing a haploid tissue and then doubling the chromosome content without accompanying cell division, to yield a plant with the diploid number of chromosomes where each chromosome pair is comprised of two duplicated chromosomes. Therefore, a DH line normally displays little or no genetic variation between individuals for traits. Lines comprising one or more genes originally comprised in a T-DNA of the present invention in the non-recurring parent also constitute plants of the present invention.

The invention is also concerned with a method of plant oil production, comprising the steps of

i) growing a plant of the present invention such as to obtain oil-containing seeds thereof,

ii) harvesting said seeds, and

iii) extracting oil from said seeds harvested in step ii), wherein the oil has a DHA content of at least 1% by weight based on the total lipid content and/or a EPA content of at least 8% by weight based on the total lipid content.

Again preferably, the content of EPA is at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content of the oil. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the oil. As described herein, the plant of the present invention comprises, for the purposes

of such method of plant oil production, preferably comprises a T-DNA of the present invention and optionally also one or more additional parts thereof, wherein the part or parts, respectively, comprise at least one expression cassette of the T-DNA of the present invention.

The invention is also concerned with parts of plants of the present invention. The term "parts of plants" includes anything derived from a plant of the invention, including plant parts such as cells, tissues, roots, stems, leaves, non-living harvest material, silage, seeds, seed meals and pollen. Preferably such plant part comprises a T-DNA of the present invention and/or comprises a content of EPA of at least 10% by weight, even more preferably at least 13% (w/w), of the total lipid content. Also preferably, the content of DHA is at least 1.5% by weight, even more preferably at least 2% (w/w), of the total lipid content of the plant part. Parts of plants of the present invention comprising, compared to wild-type plants, elevated content of EPA and/or DHA are particularly useful also for feed purposes, e.g. for aquaculture feed, e.g. as described in AU2011289381A and members of the patent family thereof.

As described in more detail in the examples, selection of the time of seed harvest is particularly beneficial for obtaining optimal amounts of VLC-PUFAs. As the PUFA content is inversely correlated with seed oil content, it is beneficial to harvest the seeds of plants of the present invention before maximum oil content is reached. Also, as described in the examples, the content of VLC-PUFAs changes during seed development. The invention thus provides an easy method of obtaining an oil containing a desired amount of a selected VLC-PUFA by harvesting seeds of plants of the present invention when their oil content corresponds to the desired amount of the selected VLC-PUFA or the desired composition of the oil. Thus, it is also possible to create blends of VLC-PUFA containing plant oils by mixing oils obtained by harvesting at different points in time or different degrees of seed ripening.

The plants of the present invention do not necessarily have to comprise a complete T-DNA of the present invention. As described above, by crossing (or back-crossing) methods it is possible to transfer arbitrary genetic material of one line to another line. Thus, by applying such crossing or back-crossing it is possible to transfer one or more, even all, expression cassettes comprised in a plant of the present invention (such plant comprising a T-DNA of the present invention) to another plant line, thereby losing e.g. a left or right border element (or both) and/or a spacer.

Thus, the invention also provides plants comprising genetic material hybridizing to a primer as given in Example 24.

Also, the invention provides plants comprising a heterologous nucleic acid segment inserted in its genetic material. The insertion is according to the invention in one of the below listed flanking regions or between a pair of flanking regions. Due to plant-to-plant variability, each flanking region may differ from the below indicated flanking regions by at most 10% calculated over a consecutive stretch of at least 100nt, preferably by at most 5% calculated over a consecutive stretch of at least – with increasing percentage identity more preferred - 90. 91. 92, 93, 94, 95, 96, 97, 98 or 99, preferred 100% identity for 100 nt. Even more preferably, the flanking region(s) comprise at least – with increasing percentage identity more preferred - 90. 91. 92, 93, 94, 95, 96, 97, 98 or 99, preferred 100% identity for 50 consecutive nucleotides identical to a fragment of a flanking region as given below, even more preferable the length of consecutive identical nucleotides is at least 100.

Table 176 provides an overview of all the flanking sequences of all loci obtained for each event listed in that table. In addition, event specific primers and probes are disclosed for event specific detection. The method for using those primers and probes is described in example 24.

As shown in the examples, insertions in these flanking regions have now been proven to lead to a surprisingly high production of VLC-PUFAs in seed, wherein such production is stable over many generations and under different growth conditions. Thus, insertion of other genetic material at these insertion locations also leads to a stable, high expression of inserted genes compared to insertions at other positions of the plant genome.

The invention is also concerned with establishing and optimizing efficient metabolic VLC-PUFA synthesis pathways. To this end, the invention provides a method for analysing desaturase reaction specificity, comprising the steps of

i) providing, to a desaturase, a detectably labelled molecule comprising a fatty acid moiety and a headgroup,

ii) allowing the desaturase to react on the labelled molecule, and

iii) detecting desaturation products.

The labelled molecule preferably is a fatty acid-coenzyme A or a fatty acid-phospholipid, the latter preferably being a lysophosphatidylcholine bound fatty acid. The method advantageously allows to determining desaturase headgroup preference or even headgroup specificity by detecting whether coenzyme A bound fatty acids are desaturated and/or whether phospholipid-bound fatty acids are desaturated.

Preferably the desaturase is provided as a microsomal fraction of an organism, preferably of yeast. Transgenic yeasts expressing the desaturase in question are easy to prepare and handle, and microsomal fractions comprising functional desaturases can be reliably and reproducibly prepared thereof without major burden. Microsomal fractions, particularly of

yeast, most preferably of Saccharomyces cerevisiae, also allow to convert fatty acid-coenzyme A molecules to fatty acids bound to other headgroups by using the yeast's native LPCAT. Likewise, microsomal fractions can be prepared from other cells and organisms.

Preferably the molecule is detectably labelled by including a radioactive isotope instead of a non-radioactive isotope. The isotope preferably is [14C]. Such label is easy to detect, does not interefere in biochemical reactions and can be incorporated in virtually any carbon-containing molecule, thereby allowing sensitively detecting and characterizing any desaturation product.

The fatty acid moiety preferably is a PUFA moiety, more preferably a VLC-PUFA moiety and most preferably a VLC-PUFA moiety. This way, the headgroup preference or specificity for economically important desaturases can be determined without having to resort to error prone and laborious feeding of live organisms or living cells.

The desaturase is allowed to react on the labelled molecule. If the desaturase can accept the labelled molecule as a substrate, then the desaturation reaction is performed. Preferably, the method is repeated by including, as a positive control, a labelled molecule which had been confirmed to be a substrate for the desaturase.

Detection preferably is accomplished using chromatography, most preferably thin layer chromatography. This technique is well known to the skilled person, readily available, very sensitive and allows differentiating even between very similar molecules. Thus, even if the positive control molecule is similar to the molecule of interest, a clear detection of desaturation products (if desaturation of the molecule occurred) is still possible.

The above method allows preparing a collection of specificity data for each desaturase, type of microsomal fraction (e.g. from yeast, plant cells etc.), fatty acid moiety and headgroup. Thus, the method can be used to select a desaturase for a given need, e.g. to accept CoA-bound fatty acids in plant cells for further presentation to an elongase. The method also allows establishing substrate specificty of the desaturase in an organism or organ of interest, e.g. a yeast, a plant leaf cell or a plant seed cell.

The invention also provides a method for analysing elongase reaction specificity, comprising the steps of

i) providing, to an elongase, a detectably labelled elongation substrate and a molecule to be elongated,

ii) allowing the elongase to elongate the molecule to be elongated using the labelled elongation substrate, and

iii) detecting elongation products.

Unless stated otherwise, the method for analysing elongase reaction specificity is performed corresponding to the method for analysing desaturase reaction specificity for corresponding reasons. The elongation substrate preferably is malonyl-CoA. The elongation substrate is preferably labelled radioactively, most preferably [14C] malonyl-CoA. Radioactive labelling allows for an easy and sensitive detection of elongation products. Also, labelling of the elongation substrate instead of the molecule to be elongated allows presenting a mixture of molecules to be elongated to the elongase, and only the elongation products will have incorporated significant amounts of label to render them easily detectable. Thus, in a single reaction vessel a multitude of potential molecules to be elongated can be assayed to determine which of these molecules are indeed elongated, and the relative affinity of the elongase to the respective molecule.

By combining both methods, it is possible to analyse even complex sequences of desaturation and elongation reactions. The invention thus also provides a method for pathway optimization, comprising the steps of

i) providing enzymes of a metabolic pathway and one or more substrates to be used by the first enzyme or enzymes of the pathway,

ii) reacting the enzymes and the substrates to produce products, which in turn are also exposed as potential substrates to the enzymes of the pathway, and

iii) determining the accumulation of products.

The method particularly allows providing desaturases and elongases to form a pathway. This is useful to determine the yield of product(s) of the pathway and of any unwanted side products. Also, by providing two or more different enzymes which perform the same metabolic function, e.g. a particular desaturation step, e.g. a delta-5 desaturation, it is possible to analyse if the presence of more than one type of enzymes has an effect on product formation, particularly on product formation rate. For such analysis, one would compare the results with a method performed with only one of the at least two enzymes. Thus, if addition of enzymes performing the same metabolic function leads to an increased yield or produc formation rate, then this metabolic step is subject to a gene dosage effect. To optimize the pathway in an organism one would correspondingly strive to implement the pathway step using the two or more enzymes as required.

Also, the method advantageously allows determining the mode of action of an enzyme in question. To this end, a helper enzyme is provided to produce a substrate for a target enzyme. The helper enzyme's mode of action is known. The helper enzyme is then provided with a substrate to turn into a product which could be used as a substrate of the target enzyme. Generation of product by the target enzyme is determined, preferably by measuring the amount of product per time or the final amount of product divided by the amount of substrate converted by the helper enzyme. Then the method is repeated using a helper enzyme of a different mode of action, and generation of product by the target

enzyme is determined, too. By comparing the product generation by the target enzyme for each mode of action, the mode of action of the target enzyme is defined as being the mode of action of the helper enzyme giving rise to the most intense generation of product by the target enzyme.

For example, to determine the mode of action of a target desaturase, a helper elongase is provided for which it has been text book knowledge that it is utilizing acyl CoA substrate and produces acyl-CoA products. Then, product generation by the target desaturase is measured. In another step, a helper desaturase is provided for which it has been established that it produces phosphatidylcholine-bound fatty acids. Again, product generation by the target desaturase is measured. When comparing the product generation of the target desaturase, the target desaturase can be defined as being a CoA-dependent desaturase if the product generation of the target desaturase under conditions where CoA-bound fatty acids are provided by the helper enzyme is more intense (e.g. higher conversion efficiency) than under conditions where phosphatidylcholine-bound fatty acids are provided by the helper enzyme.

Thus, the invention also provides a method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to produce a substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

ii) providing a non-CoA dependent desaturase to produce the substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

iii) comparing the target desaturase conversion efficiencies of step i) and ii).

If the conversion efficiency of the target desaturase is larger in step i) than in step ii), then the target desaturase is CoA-dependent. Of course, both steps must be performed under comparable conditions; particularly a substrate limitation of the target desaturase must be avoided.

The method can also be performed by providing an elongase which uses the substrate of the target desaturase. Thus, the invention provides a method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to elongate the products of the target desaturase, and determining conversion efficiency of the elongase,

ii) providing the elongase to elongate the products of a comparison desaturase known to be non-CoA dependent, and determining conversion efficiency of the elongase,

iii) comparing the elongase conversion efficiencies of step i) and ii).

If the elongase conversion efficiency is higher in step i) than in step ii), the target desaturase is CoA-dependence. Without being bound by any particular theory it is presently

expected that in such case the desaturation product does not have to be converted into an elongatable CoA-bound fatty acid, thus desaturated product can be immediately utilized by the elongase without accumulation. Of course, both steps must be performed under comparable conditions; particularly a substrate limitation of the elongase must be avoided.

The present invention also relates to oil comprising a polyunsaturated fatty acid obtainable by the aforementioned methods.

The term "oil" refers to a fatty acid mixture comprising unsaturated and/or saturated fatty acids which are esterified to triglycerides. Preferably, the triglycerides in the oil of the invention comprise PUFA or VLC-PUFA moieties as referred to above. The amount of esterified PUFA and/or VLC-PUFA is, preferably, approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. The oil may further comprise free fatty acids, preferably, the PUFA and VLC-PUFA referred to above. For the analysis, the fatty acid content can be, e.g., determined by GC analysis after converting the fatty acids into the methyl esters by transesterification. The content of the various fatty acids in the oil or fat can vary, in particular depending on the source. The oil, however, shall have a non-naturally occurring composition with respect to the PUFA and/or VLC-PUFA composition and content. It is known that most of the fatty acids in plant oil are esterified in triacylglycerides. Accordingly, in the oil of the invention, the PUFAs and VLC-PUFAs are, preferably, also occur in esterified form in the triacylglcerides. It will be understood that such a unique oil composition and the unique esterification pattern of PUFA and VLC-PUFA in the triglycerides of the oil shall only be obtainable by applying the methods of the present invention specified above. Moreover, the oil of the invention may comprise other molecular species as well. Specifically, it may comprise minor impurities of the polynucleotide or vector of the invention. Such impurities, however, can be detected only by highly sensitive techniques such as PCR.

As described above, these oils, lipids or fatty acids compositions, preferably, comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Preferred VLC-PUFAs present in the fatty acid esters or fatty acid mixtures is, preferably, at least 5,5% to 20% of DHA and/or 9,5% to 30% EPA based on the total fatty acid content.

Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention, preferably, comprise further fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10- methyleneoctadec-9-enoic acid), malvalic acid (S,9-methyleneheptadec-S-enoic acid), chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12- epoxyoctadeca-9,11-dienoic acid),

vernonic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen- 9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-S-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13, 14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12toctadecadienoic acid, calendulic acid (St10t12c-octadecatrienoic acid), catalpic acid (9t11 t13c-octadecatrienoic acid), eleostearic acid (9c11 t13t-octadecatrienoic acid), jacaric acid (Sc10t12c-octadecatrienoic acid), punicic acid (9c11 t13c-octadecatrienoic acid), parinaric acid (9c11 t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c, 11t-octadecadienoic acid). The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1 %, based on the total fatty acids, or no butter butyric acid, no cholesterol, no clupanodonic acid (= dpcpsapentaenoic acid, C22:5M,8,12,15,21) and no nisinic acid (tetracosahexaenoic acid, C23:6 L13,8,12,15,18,21).

The oils, lipids or fatty acids according to the invention, preferably, comprise at least 5.5%, 6%, 7% or 7,5%, more preferably, at least 8%, 9%, 10%, 11% or 12%, and most preferably at least 13%,14%,15%,16%. 17%, 18%, 19% or 20% of DHA, or at least 9.5%, 10%, 11% or 12%, more preferably, at least 13%, 14%, 14,5%, 15% or 16%, and most preferably at least 17%,18%,19%,20%. 21%, 22%, 23%, 24%, 25%, 26%, 27%, 28%, 29% or 30% of EPA based on the total fatty acid content of the production host cell, organism, advantageously of a plant, especially of an oil crop such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean, sunflower or the abovementioned other monocotyledonous or dicotyledonous oil crops.

A further embodiment according to the invention is the use of the oil, lipid, fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, dietary supplies, cosmetics or pharmaceutical compositions as set forth in detail below. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin such as, for example, fish oils.

The term "composition" refers to any composition formulated in solid, liquid or gaseous form. Said composition comprises the compound of the invention optionally together with suitable auxiliary compounds such as diluents or carriers or further ingredients. In this context, it is distinguished for the present invention between auxiliary compounds, i.e. compounds which do not contribute to the effects elicited by the compounds of the present invention upon application of the composition for its desired purpose, and further ingredients, i.e. compounds which contribute a further effect or modulate the effect of the compounds of the present invention. Suitable diluents and/or carriers depend on the purpose for which the composition is to be used and the other ingredients. The person

skilled in the art can determine such suitable diluents and/or carriers without further ado. Examples of suitable carriers and/or diluents are well known in the art and include saline solutions such as buffers, water, emulsions, such as oil/water emulsions, various types of wetting agents, etc.

In a more preferred embodiment of the oil-, fatty acid or lipid-containing composition, the said composition is further formulated as a pharmaceutical composition, a cosmetic composition, a foodstuff, a feedstuff, preferably, fish feed or a dietary supply.

The term "pharmaceutical composition" as used herein comprises the compounds of the present invention and optionally one or more pharmaceutically acceptable carrier. The compounds of the present invention can be formulated as pharmaceutically acceptable salts. Acceptable salts comprise acetate, methylester, Hel, sulfate, chloride and the like. The pharmaceutical compositions are, preferably, administered topically or systemically. Suitable routes of administration conventionally used for drug administration are oral, intravenous, or parenteral administration as well as inhalation. However, depending on the nature and mode of action of a compound, the pharmaceutical compositions may be administered by other routes as well. For example, polynucleotide compounds may be administered in a gene therapy approach by using viral vectors or viruses or liposomes.

Moreover, the compounds can be administered in combination with other drugs either in a common pharmaceutical composition or as separated pharmaceutical compositions wherein said separated pharmaceutical compositions may be provided in form of a kit of parts. The compounds are, preferably, administered in conventional dosage forms prepared by combining the drugs with standard pharmaceutical carriers according to conventional procedures. These procedures may involve mixing, granulating and compressing or dissolving the ingredients as appropriate to the desired preparation. It will be appreciated that the form and character of the pharmaceutically acceptable carrier or diluent is dictated by the amount of active ingredient with which it is to be combined, the route of administration and other well-known variables. The carrier(s) must be acceptable in the sense of being compatible with the other ingredients of the formulation and being not deleterious to the recipient thereof. The pharmaceutical carrier employed may be, for example, a solid, a gel or a liquid. Exemplary of solid carriers are lactose, terra alba, sucrose, talc, gelatin, agar, pectin, acacia, magnesium stearate, stearic acid and the like. Exemplary of liquid carriers are phosphate buffered saline solution, syrup, oil such as peanut oil and olive oil, water, emulsions, various types of wetting agents, sterile solutions and the like. Similarly, the carrier or diluent may include time delay material well known to the art, such as glyceryl mono-stearate or glyceryl distearate alone or with a wax. Said suitable carriers comprise those mentioned above and others well known in the art, see, e.g., Remington's Pharmaceutical Sciences, Mack Publishing Company, Easton, Pennsylvania. The diluent(s) is/are selected so as not to affect the biological activity of the

combination. Examples of such diluents are distilled water, physiological saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like. A therapeutically effective dose refers to an amount of the compounds to be used in a pharmaceutical composition of the present invention which prevents, ameliorates or treats the symptoms accompanying a disease or condition referred to in this specification. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD50/ED50. The dosage regimen will be determined by the attending physician and other clinical factors; preferably in accordance with anyone of the above described methods. As is well known in the medical arts, dosages for anyone patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Progress can be monitored by periodic assessment. A typical dose can be, for example, in the range of 1 to 1000 µg; however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the regimen as a regular administration of the pharmaceutical composition should be in the range of 1 l-lgto 10 mg units per day. If the regimen is continuous infusion, it should also be in the range of 1 l-lg to 10 mg units per kilogram of body weight per minute, respectively. Progress can be monitored by periodic assessment. However, depending on the subject and the mode of administration, the quantity of substance administration may vary over a wide range. The pharmaceutical compositions and formulations referred to herein are administered at least once in order to treat or ameliorate or prevent a disease or condition recited in this specification. However, the said pharmaceutical compositions may be administered more than one time, for example from one to four times daily up to a non-limited number of days. Specific pharmaceutical compositions are prepared in a manner well known in the pharmaceutical art and comprise at least one active compound referred to herein above in admixture or otherwise associated with a pharmaceutically acceptable carrier or diluent. For making those specific pharmaceutical compositions, the active compound(s) will usually be mixed with a carrier or the diluent, or enclosed or encapsulated in a capsule, sachet, cachet, paper or other suitable containers or vehicles. The resulting formulations are to be adopted to the mode of administration, i.e. in the forms of tablets, capsules, suppositories, solutions, suspensions or the like. Dosage recommendations shall be indicated in the prescribers or users instructions in order to anticipate dose adjustments depending on the considered recipient.

The term "cosmetic composition" relates to a composition which can be formulated as described for a pharmaceutical composition above. For a cosmetic composition, likewise, it

is envisaged that the compounds of the present invention are also, preferably, used in substantially pure form. Impurities, however, may be less critical than for a pharmaceutical composition. Cosmetic compositions are, preferably, to be applied topically.

Preferred cosmetic compositions comprising the compounds of the present invention can be formulated as a hair tonic, a hair restorer composition, a shampoo, a powder, a jelly, a hair rinse, an ointment, a hair lotion, a paste, a hair cream, a hair spray and/or a hair aerosol.

Seeds of two events described in detail in the examples section below have been deposited at ATCC under the provisions of the Budapest treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure, i.e. seeds of event "LBFLFK" = ATCC Designation "PTA-121703" and seeds of event "LBFDHG" = ATCC designation "PTA-121704". Applicants have no authority to waive any restrictions imposed by law on the transfer of biological material or its transportation in commerce. Applicants do not waive any infringement of their rights granted under this patent or rights applicable to the deposited events under the Plant Variety Protection Act (7 USC sec. 2321, et seq.), Unauthorized seed multiplication prohibited. This seed may be regulated according to national law. The deposition of seeds was made only for convenience of the person skilled in the art and does not constitute or imply any confession, admission, declaration or assertion that deposited seed are required to fully describe the invention, to fully enable the invention or for carrying out the invention or any part or aspect thereof. Also, the deposition of seeds does not constitute or imply any recommendation to limit the application of any method of the present invention to the application of such seed or any material comprised in such seed, e.g. nucleic acids, proteins or any fragment of such nucleic acid or protein.

The invention also provides a commercially relevant source of plant material, preferably of seed. As such, the invention provides a heap of at least 5kg, preferably of at least 10 kg, more preferably of at least 50kg, even more preferably of at least 100 kg, even more preferably of at least 500 kg, even more preferably of at least 1t, even more preferably of at least 2t, even more preferably of at least 5t of plant material, wherein the plant material comprises VLC-PUFAs as described according to the invention. As described herein, it is a merit of the present invention to provide, for the first time, an agronomically reliable source of VLC-PUFA plant oil, and to this end such heap of plant material is provided. The plant material is preferably plant seed, even more preferably seed of VLC-PUFA producing seed, such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of EPA is at least 10% (w/w) and/or the content of DHA is at least 1% (w/w) of the total seed fatty acid content at an oil content of 30% (w/w), or meal of such plant seed. Also, the invention provides a container comprising such plant seed in an amount of at least 5kg, preferably of at least 10 kg, more preferably of at least 50kg, even more preferably of at least 100 kg,

even more preferably of at least 500 kg, even more preferably of at least 1t, even more preferably of at least 2t, even more preferably of at least 5t. The invention thus demonstrates that the invention has well eclipsed anecdotal findings of lab scale VLC-PUFA containing plants, and instead has overcome the additional requirements for providing, on a large scale, a reliable source for VLC-PUFA and particularly for EPA and/or DHA in plant oil and plant material.

The present invention allows for the generation of plants comprising a modified fatty acid composition (as compared to control plants). Thus, the T-DNAs, the expression cassettes, vectors, polynucleotides (in particular the combination of polynucleotides), and polypeptides ((in particular the combination of polynucleotides) as disclosed herein can be used for modifying the fatty acid composition of a plant. In an embodiment, the content of at least one fatty acid disclosed in table 18 or 181 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one fatty acid disclosed in table 18 or 181 is modified in in the monoacylgylcerol (MAG) fraction, the diacylgylcerol (DAG) fraction, the triacylgylcerol (TAG) fraction, phosphatidylcholine (PC) fraction and/or phosphatidylethanolamine (PE) fraction of seed oil (increased or decreased as compared to the content of seed oil of a control plant). In another embodiment, the content of at least one Lysophosphatidylcholin species shown in Table 189 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Phosphatidylethanolamine species shown in Table 190 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Lysophosphatidylethanolamine species shown in Table 191 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant). In another embodiment, the content of at least one Triacylglycerol species shown in Table 192 is modified in seed oil (increased or decreased as compared to the content in seed oil of a control plant).

The invention is further described by means of accompanying examples and figures, which, however, are not intended to limit the scope of the invention described herein.

EXAMPLES

Example 1: General cloning methods

Cloning methods as e.g. use of restriction endonucleases to cut double stranded DNA at specific sites, agarose gel electrophoreses, purification of DNA fragments, transfer of nucleic acids onto nitrocellulose and nylon membranes, joining of DNA-fragments, transformation of *E. coli* cells and culture of bacteria were performed as described in Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87965-309-6). Polymerase chain reaction was performed using Phusion™ High-Fidelity DNA Polymerase

(NEB, Frankfurt, Germany) according to the manufactures instructions. In general, primers used in PCR were designed such, that at least 20 nucleotides of the 3' end of the primer anneal perfectly with the template to amplify. Restriction sites were added by attaching the corresponding nucleotides of the recognition sites to the 5' end of the primer. Fusion PCR, for example described by K. Heckman and L. R. Pease, Nature Protocols (2207) 2, 924–932 was used as an alternative method to join two fragments of interest, e.g. a promoter to a gene or a gene to a terminator. Gene Synthesis, as for example described by Czar et al. (Trends in Biotechnology, 2009, 27(2): 63-72), was performed by Life Technologies using their Geneart® service. The Geneart® technology, described in WO2013049227 allows production of genetic elements of a few basepair (bp) in length, and was used in this invention to produce entire plasmids of about 60,000bp. Chemical synthesis of nucleotides to polynucleotides was employed for short DNA fragments, which were then combined in a sequential, modular fashion to fragments of increasing size using a combination of conventional cloning techniques as described in WO2013049227.

Example 2: Different types of plant transformation plasmids suitable to transfer of multiple expression cassettes encoding multiple proteins into the plant genome.

For agrobacteria based plant transformation, DNA constructs preferably meet a number of criteria: (1) The construct carries a number of genetic elements that are intended to be inserted into the plant genome on a so called Transfer DNA (T-DNA) between a 'T-DNA Left Border' (LB) and 'T-DNA Right Border' (2) The construct replicates in *E.coli*, because most cloning steps require DNA multiplication steps in *E.coli*. (3) The construct replicates in *Agrobacterium* (e.g. *A. tumefaciens* or *A. rhizogenes*), because the plant transformation methods rely on using *Agrobacterium* to insert the genetic elements of interest into the plant genome of a cell that was infected by *Agrobacterium*. (4) The construct contains supporting genetic elements that encode proteins which are required for infection of the plant cell, and for transfer and integration of desired genetic elements into the plant genome of an plant cell infected by the *Agrobacterium*, or the construct was used in combination with a second construct containing such supporting genetic elements that was present in the same Agrobacterium cell. (5) The constructs can contain selection markers to facilitate selection or identification of bacterial cells that contain the entire construct, and of a plant cell(s) that contains the desired genetic elements. An overview of available plasmids was given in Komori et al (2007).

Agrobacteria mediated transformation results in an almost random integration (with some bias induced by a number of factors) of the desired genetic element into chromosomes of the plant cell. The goal of the transformation was to integrate the entire T-DNA from T-DNA

Left border to T-DNA Right border into a random position of a random chromosome. It can also be desirable to integrate the entire T-DNA twice or three times into the genome, for example to increase the plant expression levels of genes encoded by the T-DNA. To avoid complex Mendelian segregation of multiple integrations, it was preferred to have all T-DNA insertions at one genomic location, ('locus'). Inserting more than 25,000 bp T-DNA into plant genomes has been found to be a particular challenge in the current invention. In particular, it has been found in this invention plasmids carrying a ColE1/pVS1 origin of replication for plasmid replication in *E.coli* and/or *Agrobacterium*, are not stable above ~25,000 bp. Such plasmids of the invention are described in Example 3. Because of this limitation, not more than ~4 to 5 gene expression cassettes can be transferred on one T-DNA containing plasmid into the plant genome. However, for the current invention up to 13 gene expression cassettes having a combined size of about 44,000 bp needed to be transferred into the plant genome. In contrast to plasmids containing the ColE1/pVS1 origin of replication for high copy plasmid replication in *E.coli* and/or *Agrobacterium*, BiBAC plasmids (Hammilton 1997) containing the F factor / pRi origin of replication for single copy plasmid replication in *E.coli* and/or *Agrobacterium* where found to be stable in this invention up to a size of ~60,000 bp. Such plasmids of the invention are described in Example 4. Both approaches described above were followed in the current invention.

Example 3: Assembly of genes required for EPA and DHA synthesis within T-plasmids containing the ColE1/pVS1 origin of replication

For synthesis of VLC-PUFA in *Brassica napus* seeds, the set of genes encoding the proteins of the metabolic VLC-PUFA pathway were combined with expression elements (promoter, terminators, Introns) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants. Attributed to the large number of expression cassettes promoting expression of one protein each, two binary t-plasmids T-DNA where used for cloning of the complete set of proteins required for EPA and DHA synthesis. To this end, the general cloning strategy depicted in Figure 3 was employed. While Figure 3 depicts the general strategy, cloning of the final plant expression vectors described in example 10 to 14 was not restricted to this strategy; specifically a combination of all methods known to one skilled in the art, such as cloning, the use of restriction endonucleases for generation of sticky and blunt ends, synthesis and fusion PCR has been used. Following the modular cloning scheme depicted in Figure 3, genes were either synthesized by GeneArt (Regensburg) or PCR-amplified using Phusion™ High-Fidelity DNA Polymerase (NEB, Frankfurt, Germany) according to the manufactures instructions from cDNA. In both cases an *Nco* I and/or *Asc* I restriction site at the 5' terminus, and a

Pac I restriction site at the 3' terminus (Figure 3A) were introduced to enable cloning of these genes between functional elements such as promoters and terminators using these restriction sites (see below in this example). Promoter-terminator modules or promoter-intron-terminator modules were created by complete synthesis by GeneArt (Regensburg) or by joining the corresponding expression elements using fusion PCR as described in example 1 and cloning the PCR-product into the TOPO-vector pCR2.1 (Invitrogen) according to the manufactures instructions (Figure 3B). While joining terminator sequences to promoter sequences or promoter-intron sequences either via synthesis of whole cassettes or using fusion PCR, recognition sequences for the restriction endonucleases depicted in Figure 3 were added to either side of the modules, and the recognition sites for the restriction endonucleases *Nco* I, *Asc* I and *Pac* I were introduced between promoter and terminator or between introns and terminator (see Figure 3B). To obtain the final expression modules, PCR-amplified genes were cloned between promoter and terminator or intron and terminator via *Nco* I and/or *Pac* I restriction sites (Figure 3C). Employing the custom multiple cloning site (MCS) up to three of those expression modules were combined as desired to expression cassettes harbored by either one of pENTR/A, pENTR/B or pENTR/C (Figure 3D). Finally, the Multi-site Gateway™ System (Invitrogen) was used to combine three expression cassette harbored by pENTR/A, pENTR/B and pENTR/C (Figure 3E) to obtain the final binary pSUN T-plasmids for plant transformation VC-LJB2197-1qcz, VC-LJB2755-2qcz rc, VC-LLM306-1qcz rc, VC-LLM337-1qcz rc, VC-LLM338-3qcz rc and VC-LLM391-2qcz rc. An overview of binary vectors and their usage was given by Hellens *et al*, Trends in Plant Science (2000) 5: 446–451.

The structure of the plamsids VC-LJB2197-1qcz, VC-LJB2755-2qcz rc, VC-LLM306-1qcz rc, VC-LLM337-1qcz rc, VC-LLM338-3qcz rc, VC-LLM391-2qcz rc, and VC-LTM217-1qcz rc was given in the Table 1, Table 2, Table 3, Table 4, Table 5, Table 6, and Table 7

Nomeclature of genetic elements:

- j- indicates a junction between two genetic elements
- c- coding sequence
- t- terminator
- p- promotor
- i- intron

T-DNA Transferred DNA

- RB Right Border of the T-DNA
- LB Left Border of the T-DNA

Table 1 Genetic Elements of plasmid VC-LJB2197-1qcz. Listed are the names of the elements, the position in VC-LJB2197-1qcz (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LJB2197-1qcz), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LJB2197-1qcz) and a left border (nucleotides 22232 to 22105 of VC-LJB2197-1qcz). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377[LJK32]	3512	3888	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene OCS from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of

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Genetic Elements of plasmid VC-LJB2197-1qcz	From	To	Description, Function and Source of Element
			transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377[LJK32]	14207	14583	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
t-AtAHAS-3'/UTR[bb003]	16576	17790	Terminator from *AtAHASL* [*csr1-2*] of *acetohydroxyacid synthase* gene from *Arabidopsis*
p-PcUbi4-2[long]	17823	18804	Promoter from *UBIQUITIN* gene *UBI4-2* with internal intron from *Petroselinum crispum*
c-AtAHASL_A122T_S653N	18812	20824	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS with S653N(*csr1-2*) mutation and A122T SDM mutation from *Arabidopsis*
t-AtAHAS-3'/UTR[ac321]	20849	22064	Terminator from *AtAHASL* [*csr1-2*] of *ACETOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
b-JTV_LB	22232	22105	Left border of T-DNA
c-StaA[lm500]	22338	22967	PVS1 partitioning protein
c-VS1orf3[lm500]	23203	22988	*VS1*orf3
c-repA[lm500]	23294	24469	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	24535	24729	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	25032	24830	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	25451	25171	pBR322 origin of replication [ecoli] from AF234316 pCambia2301
c-aadAmod1	26588	25797	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	26767	26589	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

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Table 2: Genetic Elements of plasmid VC-LJB2755-2qcz rc. Listed are the names of the elements, the position in VC-LJB2755-2qcz rc (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LJB2755-2qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LJB2755-2qcz rc) and a left border (nucleotides 26117 to 25990 of VC-LJB2755-2qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
b-RB[Im175]	148	4	Right border of T-DNA
p-LuPXR 1727bp[LLL823]	342	2068	Promoter from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	2069	2826	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	2842	3933	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	3990	4389	Terminator from *PEROXIREDOXIN LIKE PROTEIN* gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	4468	5531	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377[LJK32]	5532	5908	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	5912	7231	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	7232	7423	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	7559	9357	Promoter from a *SUCROSE BINDING RELATED-PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	9358	9812	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	9822	11192	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	11220	11454	Terminator from *CATHEPSIN D INHIBITOR* gene [CATHD] from *Solanum tuberosum* [Potato]
p-BnFae1	11533	12962	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica*

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Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
			napus
i-Atss1_847bp[LJK19]	12963	13809	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	13812	14630	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-bnFae1	14646	15045	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-Napin A/B	15166	15829	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377[LJK32]	15830	16206	i-Atss14_377bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA)	16213	17409	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	17428	17985	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-VfUSP_684bp[LLL894]	18064	18747	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	18748	18999	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	19010	20095	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	20104	20319	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
t-AtAHAS-3'/UTR[ac321]	20460	21675	Terminator from *AtAHASL* [*csr1-2*] of *ACETYOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
p-PcUbi4-2[long]	21708	22689	Promoter from *UBIQUITIN* gene *UBI4-2* with internal intron from *Petroselinum crispum*
c-AtAHASL_A122T_S653N	22697	24709	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS with S653N(*csr1-2*) mutation and A122T SDM mutation from *Arabidopsis*
t-AtAHAS-3'/UTR[ac321]	24734	25949	Terminator from *AtAHASL* [*csr1-2*] of *ACETOHYDROXYACID SYNTHASE* gene from *Arabidopsis*
b-JTV_LB	26117	25990	Left border of T-DNA
c-StaA[lm500]	26223	26852	PVS1 partitioning protein

Genetic Elements of plasmid VC-LJB2755-2qcz rc	From	To	Description, Function and Source of Element
c-VS1orf3[lm500]	27088	26873	*VS1*orf3
c-repA[lm500]	27179	28354	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	28420	28614	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	28917	28715	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	29336	29056	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	30473	29682	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	30652	30474	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 3: Genetic Elements of plasmid VC-LLM306-1qcz rc. Listed are the names of the elements, the position in VC-LLM306-1qcz rc (note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM306-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM306-1qcz rc) and a left border (nucleotides 20180 to 20053 of VC-LLM306-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM306-1qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-LuCnl(1064bp)	342	1405	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	1416	3041	*Delta 4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	3063	3254	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFAE1	3448	4877	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	4878	5724	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	5732	6634	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFAE1	6651	7050	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-VfSBP_perm3	7099	8897	Promoter derived from a promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*

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Genetic Elements of plasmid VC-LLM306-1qcz rc	From	To	Description, Function and Source of Element
c-o3Des(Pir_GA)	8901	9992	*Omega-3 DESATURASE* from *Pythium irregulare*
t-StCATHD-pA	10023	10257	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-VfUSP_684bp[LLL894]	10331	11014	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	11015	11772	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	11789	12874	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	12924	13139	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	13259	14409	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)	14420	15979	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	15993	16592	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-BnFae1	16671	18100	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
c-d6Des(Ot_febit)	18109	19479	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-bnFae1	19493	19892	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
b-JTV_LB	20180	20053	Left border of T-DNA
c-StaA[lm500]	20286	20915	PVS1 partitioning protein
c-VS1orf3[lm500]	21151	20936	*VS1*orf3
c-repA[lm500]	21242	22417	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	22483	22677	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	22980	22778	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	23399	23119	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	24536	23745	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	24715	24537	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 4: Genetic Elements of plasmid VC-LLM337-1qcz rc. Listed are the names of the elements, the position in VC-LLM337-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM337-1qcz rc), the function and source of the element.

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The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM337-1qcz rc) and a left border (nucleotides 16953 to 16826 of VC-LLM337-1qcz rc. Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM337-1qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-LuCnl(1064bp)	342	1405	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	1416	3041	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	3063	3254	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	3448	4877	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	4878	5724	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	5732	6634	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	6651	7050	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-VfSBP_perm3	7099	8897	Promoter derived from a promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
c-o3Des(Pir_GA)	8901	9992	*Omega-3 DESATURASE* from *Pythium irregulare*
t-StCATHD-pA	10023	10257	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-VfUSP_684bp[LLL894]	10331	11014	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	11015	11772	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	11789	12874	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	12924	13139	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	13259	14409	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*

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Genetic Elements of plasmid VC-LLM337-1qcz rc	From	To	Description, Function and Source of Element
c-d4Des(Tc_GA)	14420	15979	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	15993	16592	Terminator of *ARC5* gene from *Phaseolus vulgaris*
b-JTV_LB	16953	16826	Left border of T-DNA
c-StaA[lm500]	17059	17688	PVS1 partitioning protein
c-VS1orf3[lm500]	17924	17709	*VS1*orf3
c-repA[lm500]	18015	19190	pVS1 replication protein [repA] gene/CDS
o-pVS1-origin	19256	19450	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	19753	19551	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	20172	19892	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	21309	20518	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	21488	21310	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 5: Genetic Elements of plasmid VC-LLM338-3qcz rc. Listed are the name of the element, the position in VC-LLM338-3qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand VC-LLM338-3qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM338-3qcz rc) and a left border (nucleotides 17069 to 16942 of VC-LLM338-3qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-VfSBP_perm3	341	2139	Promoter derived from a promoter from a *SUCROSE-BINDING RELATED-PROTEIN* gene from *Vicia faba*
c-d15Des(Ch_ERTp_GA)	2151	3389	*Delta-15 DESATURASE* from *Cochliobolus heterostrophus* C5
t-StCATHD-pA	3420	3654	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-BnFae1	3848	5277	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*

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Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
i-Atss1_847bp[LJK19]	5278	6124	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	6132	7034	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	7051	7450	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-VfUSP_684bp[LLL894]	7529	8212	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss15_758bp[LJK33]	8213	8970	i-Atss15_758bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-c3Des(Pi_GA2)	8987	10072	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	10122	10337	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-ARC5_perm1	10457	11607	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)	11618	13177	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	13191	13790	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-LuCnl(1064bp)	13869	14932	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	14943	16568	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	16590	16781	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	17069	16942	Left border of T-DNA
c-StaA[lm500]	17175	17804	PVS1 partitioning protein
c-VS1orf3[lm500]	18040	17825	*VS1*orf3
c-repA[lm500]	18131	19306	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	19372	19566	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	19869	19667	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	20288	20008	pBR322 origin of replication [*E. Coli*] from AF234316 pCambia2301
c-aadAmod1	21425	20634	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100

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Genetic Elements of plasmid VC-LLM338-3qcz rc	From	To	Description, Function and Source of Element
p-aadA[lm800]	21604	21426	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 6: Genetic Elements of plasmid VC-LLM391-2qcz rc rc. Listed are the names of the elements, the position in VC-LLM391-2qcz rc rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLM391-2qcz rc rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLM391-2qcz rc rc) and a left border (nucleotides 10947 to 10820 of VC-LLM391-2qcz rc rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LLM391-2qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-BnFae1	540	1969	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	1970	2816	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	2824	3726	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	3743	4142	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5perm1	4335	5485	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA)_T564G	5496	7055	*Delta-4 DESATURASE* from *Thraustochytrium* spp.
t-pvarc	7069	7668	Terminator of *Arc5* gene from *Phaseolus vulgaris*
p-LuCnl(1064bp)	7747	8810	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Eg_GA)	8821	10446	*Delta-4 DESATURASE* from *Euglena gracilis*
t-AgrOCS 192bp[LED12]	10468	10659	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	10947	10820	Left border of T-DNA
c-StaA[lm500]	11053	11682	PVS1 partitioning protein

Genetic Elements of plasmid VC-LLM391-2qcz rc	From	To	Description, Function and Source of Element
c-VS1orf3[lm500]	11918	11703	*VS1*orf3
c-repA[lm500]	12009	13184	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	13250	13444	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	13747	13545	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	14166	13886	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	15303	14512	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	15482	15304	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Table 7: Genetic Elements of plasmid VC-LLTM217-1qcz rc. Listed are the names of the elements, the position in VC-LLTM217-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LLTM217-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 148 to 4 of VC-LLTM217-1qcz rc) and a left border (nucleotides 10659 to 10532 of VC-LLTM217-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in E.coli and/or agrobacteria.

Genetic Elements of plasmid VC-LTM217-1qcz rc	From	To	Description, Function and Source of Element
b-RB[lm175]	148	4	Right border of T-DNA
p-BnFae1	540	1969	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	1970	2816	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	2824	3726	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	3743	4142	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	4335	5485	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*

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Genetic Elements of plasmid VC-LTM217-1qcz rc	From	To	Description, Function and Source of Element
c-d4Des(Tc_GA)	5496	7055	*Delta-4 DESATURASE* from *Thraustochytrium* spp.
t-pvarc	7069	7668	Terminator of *Arc5* gene from *Phaseolus vulgaris*
p-LuCnl(1064bp)	7747	8810	Promoter from *CONLININ* gene from *Linum usitatissimum*
c-d4Des(Pl_GA)2	8821	10158	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	10180	10371	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
b-JTV_LB	10659	10532	Left border of T-DNA
c-StaA[lm500]	10765	11394	PVS1 partitioning protein
c-VS1orf3[lm500]	11630	11415	*VS1*orf3
c-repA[lm500]	11721	12896	pVS1 replication protein [*repA*] gene/CDS
o-pVS1-origin	12962	13156	broad host-range replication origin of plasmid pVS1 (Genbank: AF133831, Itoh et al.1984)
o-ColE1-bom[lm500]	13459	13257	pBR322 bom site, partial, from AF234316 pCambia2301
o-Rep-ColE1	13878	13598	pBR322 origin of replication [*E. coli*] from AF234316 pCambia2301
c-aadAmod1	15015	14224	Codon Optimized *Adenyltransferase* [*aadA*] gene/CDS from SUN100
p-aadA[lm800]	15194	15016	*Adenyltransferase* [*aadA*] Spectinomycin Prokaryotic promoter

Example 4: Assembly of genes required for EPA and DHA synthesis within BiBAC T-plasmids containing the F factor / pRI origin of replication

For synthesis of VLC-PUFA in *Brassica napus* seeds, the set of genes encoding the proteins of the metabolic VLC-PUFA pathway were combined with expression elements (promoter, terminators and introns) and transferred into binary t-plasmids that were used for agrobacteria mediated transformation of plants. While the large number of expression cassettes promoting expression of one protein each, were distributed in example 3 onto two binary t-plasmids T-DNA, in this example all expression cassettes have been combined onto a single binary T-plasmid. The advance of DNA synthesis allows numerous companies to offer services to use a combination of chemical synthesis and molecular biological techniques to synthesize de novo, without an initial template, polynucleotides up to the size of microbial genomes. Synthesis used in the construction of the plasmids described in this example was performed by Life Technologies using their Geneart® service. The Geneart® technology, described in WO2013049227 allows production of genetic elements of a few basepair (bp) length, and was used in this invention to produce the binary T-plasmids for plant transformation VC-RTP10690-1qcz_F, VC-RTP10691-2qcz, VC-LTM595-1qcz rc and VC-LTM593-1qcz rc having a total size of ~61.000bp for each construct. The structure of the plasmids VC-RTP10690-1qcz_F, VC-RTP10691-2qcz, VC-LTM595-1qcz rc and VC-LTM593-1qcz rc is given in: Table 8, Table 9, Table 10 and Table 11.

Table 8: Genetic Elements of plasmid RTP10690-1qcz_F. Listed are the names of the elements, the position in RTP10690-1qcz_F (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of SEQ ID NO.6), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 59918 to 148 of RTP10690-1qcz_F) and a left border (nucleotides 43853 to 43718 of RTP10690-1qcz_F). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [CATHD] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16462	18188	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18189	18946	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18962	20053	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20110	20509	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	20645	21708	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss2_455bp[LJK20]	21709	22163	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d4Des(Pl_GA)2	22181	23518	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	23540	23731	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	23925	25354	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25355	26202	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
T	25513	25513	T inserted [14-T string found verses 13-T string in original i-Atss1_847bp[LJK19]
c-d5Elo(Ot_GA3)	26210	27112	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27129	27528	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27681	28831	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28842	30401	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30415	31014	Terminator of *ARC5* gene from *Phaseolus vulgaris*

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	31093	31776	Promoter from unknown seed protein gene USP (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31777	32028	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32039	33124	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33133	33348	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnSETL-v1[1234bp]	33484	34717	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	34720	36039	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	36061	36674	*SETL*-v1 *Brassica napus* terminator
p-BnSETL-v1[1234bp]	36767	38000	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	38003	39094	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	39116	39729	*SETL*-v1 *Brassica napus* terminator
p-YPC105906 PcUbi4-2[long]	39853	40829	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	40837	42849	*ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	42850	43629	*Arabidopsis* (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for *ACETOHYDROXYACID SYNTHASE* gene
b-LLB	43853	43718	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	45800	44985	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	45921	45801	Promoter for Kanamycin resistance gene
o-ori-2	47074	47290	ori-2 origin of replication
c-repE	47384	48139	*repE* gene/CDS
c-sopA	48718	49893	*sapA* gene/CDS
c-sopB	49893	50864	*sopB* gene/CDS
c-sopC/incD	50937	51410	*incD*/*sopC* partial gene/CDS
c-traI	51913	51972	*traI* gene/CDS
mf-traI - repA intergenic region	51961	52323	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	52324	53541	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,

Genetic Elements of plasmid RTP10690-1qcz_F	From	To	Description, Function and Source of Element
			Jan. 2000, p. 179..188)
rr-repB	53771	54781	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	54996	56315	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
mf-y4cG	56794	56324	fragment of DNA invertase homolog; similar to Rhizobium sp. NGR234 pNGR234a Y4CG
tr-Tn5	58834	57273	Transposon Tn5 sequence
o-oriT	59130	59298	oriT from pRK310 genbank file
b-RB[rtp4394]	148	59918	Right T-DNA Right border

Table 9: Genetic Elements of plasmid RTP10691-2qcz. Listed are the names of the elements, the position in RTP10691-2qcz (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of RTP10691-2qcz), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 60923 to 148 of RTP10691-2qcz and a left border (nucleotides 44858 to 44723 of RTP10691-2qcz). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E.coli* and/or agrobacteria.

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			Thraustochytrium sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING RELATED PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIS D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16462	18188	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18189	18946	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18962	20053	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20110	20509	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-BnSETL-v1[1234bp]	20645	21878	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc GA2)	21881	23200	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	23222	23835	*SETL*-v1 *Brassica napus* terminator
p-BnFae1	24029	25458	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25459	26302	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
delTTT	25612	25612	Three T deleted compared to original i-Atss1_847bp[LJK19]
c-d5Elo(Ot_GA3)	26310	27212	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27229	27628	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27781	28931	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28942	30501	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30515	31114	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-VfUSP_684bp[LLL894]	31193	31876	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31877	32128	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32139	33224	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33233	33448	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnFae1	33642	35071	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica*

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			napus
i-Atss1_847bp[LJK19]	35072	35918	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d4Des(Pl_GA)2	35926	37263	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-bnFae1	37280	37679	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-BnSETL-v1[1234bp]	37772	39005	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	39008	40099	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	40121	40734	*SETL*-v1 *Brassica napus* terminator
p-YPC105906_PcUbi4-2[long]	40858	41834	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	41842	43854	*ACETOHYDROXYACID SYNTHASE LARGE SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	43855	44634	Arabidopsis (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for *ACETOHYDROXYACID SYNTHASE* gene
b-LLB	44858	44723	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	46805	45990	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	46926	46806	Promoter for Kanamycin resistance gene
o-ori-2	48079	48295	ori-2 origin of replication
c-repE	48389	49144	*repE* gene/CDS
c-sopA	49723	50898	*sapA* gene/CDS
c-sopB	50898	51869	*sopB* gene/CDS
c-sopC/incD	51942	52415	*incD*/*sopC* partial gene/CDS
c-traI	52918	52977	*traI* gene/CDS
mf-traI - repA intergenic region	52966	53328	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	53329	54546	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
rr-repB	54776	55786	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	56001	57320	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,

Genetic Elements of plasmid RTP10691-2qcz	From	To	Description, Function and Source of Element
			Jan. 2000, p. 179..188)
mf-y4cG	57799	57329	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	59839	58278	Transposon Tn5 sequence
o-oriT	60135	60303	oriT from pRK310 genbank file
b-RB[rtp4394]	60923	148	Right T-DNA Right border

Table 10: Genetic Elements of plasmid VC-LTM595-1qcz rc. Listed are the names of the elements, the position in VC-LTM595-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LTM595-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 60913 to 148 of VC-LTM595-1qcz rc) and a left border (nucleotides 44848 to 44713 of VC-LTM595-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E. coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING RELATED-PROTEIN* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIS D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE*

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			protein gene *PXR* from *Linum usitatissimum*
i-Atss1_847bp[LJK19]	11240	12086	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d6Elo(Tp_GA2)	12100	12918	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12974	13373	Terminator from *PEROXIREDOXIN LIKE* protein gene *PXR* (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13543	14206	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14207	14583	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14590	15786	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15805	16362	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-LuPXR 1727bp[LLL823]	16455	18181	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	18182	18939	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	18955	20046	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	20103	20502	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-BnSETL-v1[1234bp]	20638	21871	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	21874	23193	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	23215	23828	*SETL*-v1 *Brassica napus* terminator
p-BnFae1	24022	25451	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	25452	26298	i-Atss1_847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*,

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	26306	27208	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	27225	27624	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-ARC5_perm1	27771	28921	Promoter derived from a promoter from *ARCILINE 5* gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	28932	30491	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	30505	31104	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-VfUSP_684bp[LLL894]	31183	31866	Promoter from *UNKNOWN SEED PROTEIN* gene USP (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	31867	32118	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	32129	33214	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	33223	33438	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnFae1	33632	35061	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	35062	35908	i-Atss1 847bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d4Des(Pl_GA)2	35916	37253	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-bnFae1	37270	37669	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-BnSETL-v1[1234bp]	37762	38995	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	38998	40089	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	40111	40724	*SETL*-v1 *Brassica napus* terminator
p-YPC105906_PcUbi4-2[long]	40848	41824	MTX Parsley (*Petroselinum crispum*) *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	41832	43844	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS from

Genetic Elements of plasmid VC-LTM595-1qcz rc	From	To	Description, Function and Source of Element
			Arabidopsis with S653N (*csr1-2*) mutation and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	43845	44624	*Arabidopsis* (dicot) *AtAHASL* 3' Un-uranslated Region [trimmed] terminator for ACETOHYDROXYACID SYNTHASE gene
b-LLB	44848	44713	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	46795	45980	Kanamycin Resistance selection gene/CDS
p-Kan[Im500]	46916	46796	Promoter for Kanamycin resistance gene
o-ori-2	48069	48285	ori-2 origin of replication
c-repE	48379	49134	*repE* gene/CDS
c-sopA	49713	50888	*sapA* gene/CDS
c-sopB	50888	51859	*sopB* gene/CDS
c-sopC/incD	51932	52405	*incD*/*sopC* partial gene/CDS
c-traI	52908	52967	*traI* gene/CDS
mf-traI - repA intergenic region	52956	53318	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179-188)
o-repA	53319	54536	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
rr-repB	54766	55776	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
o-repC	55991	57310	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
mf-y4cG	57789	57319	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	59829	58268	Transposon Tn5 sequence
o-oriT	60125	60293	oriT from pRK310 genbank file
b-RB[rtp4394]	148	60913	Right T-DNA Right border

Table 11: Genetic Elements of plasmid VC-LTM593-1qcz rc. Listed are the names of the elements, the position in VC-LTM593-1qcz rc (nucleotide number, note: start position was larger than stop position for elements encoded by the complementary strand of VC-LTM593-1qcz rc), the function and source of the element. The T-DNA integrated into the plant genome during the transformation process was flanked by a right border (nucleotides 59895 to 148 of VC-LTM593-1qcz rc) and a left border (nucleotides 43830 to43695 of VC-LTM593-1qcz rc). Elements outside of that region (=vector backbone) are required for cloning and stable maintenance in *E. coli* and/or agrobacteria.

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
p-VfUSP_684bp[LLL894]	329	1012	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	1013	1264	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-d6Elo(Pp_GA2)	1267	2139	*Delta-6 ELONGASE* from *Physcomitrella patens*
t-CaMV35S	2140	2355	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-LuCnl(1064bp)	2448	3511	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss14_377bp[LJK32]	3512	3888	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d5Des(Tc_GA2)	3892	5211	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-AgrOCS 192bp[LED12]	5212	5403	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-SBP	5539	7337	Promoter from a *SUCROSE-BINDING PROTEIN-RELATED* gene from *Vicia faba*
i-Atss2_455bp[LJK20]	7338	7792	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d6Des(Ot_febit)	7802	9172	*Delta-6 DESATURASE* from *Ostreococcus tauri*
t-StCATHD-pA	9200	9434	Terminator from *CATHEPSIN D INHIBITOR* gene [*CATHD*] from *Solanum tuberosum* [Potato]
p-LuPXR 1727bp[LLL823]	9513	11239	Promoter from *PEROXIREDOXIN LIKE*

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			protein gene *PXR* from *Linum usitatissimum*
i-Atss1_846bp[ltm593]	11240	12085	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); 1 bp at poly T stretch shorter compared to original i-Atss1_847bp
c-d6Elo(Tp_GA2)	12099	12917	*Delta-6 ELONGASE* from *Thalassiosira pseudonana*
t-AtPXR 400bp[LLL823]	12973	13372	Terminator from peroxiredoxin like protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-Napin A/B	13542	14205	Promoter from *napA/B* gene (napin, seed storage protein) from *Brassica napus*
i-Atss14_377bp[LJK32]	14206	14582	i-Atss14_377bp[LJK32] functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At5g63190, +166 to + 542 bp (numbering relative to start of transcription) (+201 to + 542 bp 5'UTR-Intron only)
c-d12Des(Ps_GA2)	14589	15785	*Delta-12 DESATURASE* from *Phythophthora sojae*
t-E9	15804	16361	Terminator from Small Subunit of RuBisCo *rbcS* gene (*E9*) from *Pisum sativum*
p-BnSETL-v1[1234bp]	16454	17687	*SETL*-v1 *Brassica napus* promoter
c-o3Des(Pir_GA)	17690	18781	*Omega-3 DESATURASE* from *Pythium irregulare*
t-BnSETL	18803	19416	*SETL*-v1 *Brassica napus* terminator
p-VfUSP_684bp[LLL894]	19495	20178	Promoter from *UNKNOWN SEED PROTEIN* gene *USP* (accession: X56240) from *Vicia faba*
i-Atss18_252[LJK36]	20179	20430	i-Atss18_252bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g01170, + 37 to + 288 bp (numbering relative to start of transcription) (+ 72 to + 282bp 5'UTR-Intron only)
c-o3Des(Pi_GA2)	20441	21526	*Omega-3-DESATURASE* from *Phythophthora infestans*
t-CaMV35S	21535	21750	Terminator CaMV35S from *35S* gene from Cauliflower mosaic virus
p-BnSETL-v1[1234bp]	21886	23119	*SETL*-v1 *Brassica napus* promoter
c-d5Des(Tc_GA2)	23122	24441	*Delta-5 DESATURASE* from *Thraustochytrium* sp. ATCC21685
t-BnSETL	24463	25076	*SETL*-v1 *Brassica napus* terminator
p-ARC5_perm1	25223	26373	Promoter derived from a promoter from

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			ARCILINE 5 gene from *Phaseolus vulgaris*
c-d4Des(Tc_GA3)	26384	27943	*Delta-4 DESATURASE* from *Thraustochytrium* sp.
t-pvarc	27957	28556	Terminator of *ARC5* gene from *Phaseolus vulgaris*
p-LuPXR 1727bp[LLL823]	28649	30375	Promoter from *PEROXIREDOXIN LIKE* protein gene *PXR* from *Linum usitatissimum*
i-Atss15_758bp[LJK33]	30376	31133	i-Atss15_758bp[LJK33] functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At2g27040, +93 bp to + 850 bp (numbering relative to start of transcription) (+128 to + 847 bp 5'UTR-Intron only)
c-o3Des(Pir_GA)	31149	32240	*Omega-3 DESATURASE* from *Pythium irregulare*
t-AtPXR 400bp[LLL823]	32297	32696	Terminator from *PEROXIREDOXIN LIKE* protein gene PXR (At1g48130) from *Arabidopsis thaliana*
p-LuCnl(1064bp)	32832	33895	Promoter from *CONLININ* gene from *Linum usitatissimum*
i-Atss2_455bp[LJK20]	33896	34350	i-Atss2_455bp functional intron region; intron with partial 5'UTR, *Arabidopsis thaliana*, Locus At1g65090, +77 to +531bp (numbering relative to start of transcription) (+113 to + 508bp 5'UTR-Intron only)
c-d4Des(Pl_GA)2	34360	35697	*Delta-4 DESATURASE* from *Pavlova lutheri*
t-AgrOCS 192bp[LED12]	35719	35910	Terminator from *OCTOPINE SYNTHASE* gene *OCS* from *Agrobacterium tumefaciens*
p-BnFae1	36104	37533	Promoter from *Beta-KETOACYL-CoA SYNTHASE* (*FAE1.1*) gene from *Brassica napus*
i-Atss1_847bp[LJK19]	37534	38380	i-Atss1_847bp functional intron region; intron with partial 5' UTR, *Arabidopsis thaliana*, Locus At1g62290 (aspartyl protease family protein), +1 to +847bp (numbering relative to start of transcription) (+19 to +841bp 5'UTR-Intron only); from QC1153-1/RTP6393.
c-d5Elo(Ot_GA3)	38388	39290	*Delta-5 ELONGASE* from *Ostreococcus tauri*
t-bnFae1	39307	39706	Terminator from *FATTY ACID ELONGASE* (*FAE 1.1*) gene from *Brassica juncea*
p-YPC105906_PcUbi4-2[long]	39830	40806	MTX Parsley *UBI4-2* promoter with internal intron
c-AtAHASL_A122T_S653N[minusRES]	40814	42826	*ACETOHYDROXYACID SYNTHASE LARGE-SUBUNIT* gene/CDS from *Arabidopsis* with S653N (*csr1-2*) mutation

Genetic Elements of plasmid VC-LTM593-1qcz rc	From	To	Description, Function and Source of Element
			and A122T SDM mutation minus restriction sites
t-AtAHAS-3'UTR[rtp4820]	42827	43606	*Arabidopsis* (dicot) *AtAHASL* 3' Un-translated Region [trimmed] terminator for ACETOHYDROXYACID SYNTHASE gene
b-LLB	43830	43695	Left T-DNA Left border from pTi15955 [Genbank #AF242881]
c-KanR_Tn903	45777	44962	Kanamycin Resistance selection gene/CDS
p-Kan[lm500]	45898	45778	Promoter for Kanamycin resistance gene
o-ori-2	47051	47267	ori-2 origin of replication
c-repE	47361	48116	*repE* gene/CDS
c-sopA	48695	49870	*sapA* gene/CDS
c-sopB	49870	50841	*sopB* gene/CDS
c-sopC/incD	50914	51387	*incD/sopC* partial gene/CDS
c-traI	51890	51949	*traI* gene/CDS
mf-traI - repA intergenic region	51938	52300	regulatory region of traR dependent quorum sensing regulon - containing 2 tra-boxes (see LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179-188)
o-repA	52301	53518	*Rep-A* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
rr-repB	53748	54758	*rep-B* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY, Jan. 2000, p. 179..188)
o-repC	54973	56292	*rep-C* gene from pTiC58 replicon (LI AND FARRAND JOURNAL OF BACTERIOLOGY,Jan. 2000, p. 179..188)
mf-y4cG	56771	56301	fragment of DNA invertase homolog; similar to *Rhizobium* sp. NGR234 pNGR234a Y4CG
tr-Tn5	58811	57250	Transposon Tn5 sequence
o-oriT	59107	59275	oriT from pRK310 genbank file
b-RB[rtp4394]	148	59895	Right T-DNA Right border

Table 13 compares the order of the gene expression cassettes among all the different constructs and the construct combinations, using short terms for these expression cassettes, see Table 12 for definitions. The data in Examples 10 to 19 demonstrate significant differences among the different construct or construct combinations in terms of the PUFA profile measured in transgenic seed. The differences between constructs and the construct combinations were evident even when eliminating all other sources that affect PUFA levels (e.g. different environments, plant-to-plant variability, seed oil content, T-DNA

copy number). For example VC-RTP10690-1qcz_F and VC-LMT593-1qcz rc are isogenic, i.e. the two constructs contained exactly the same gene expression cassettes. Because of the similarity between RTP10690-1qcz_F and VC-LMT593-1qcz one would expect exactly the same pathway step conversion efficiencies e.g. when comparing the average conversion efficiencies of all single copy events. However, Figure 39 shows that VC-RTP10690-1qcz_F had a Delta-4 DESATURASE conversion efficiency of 32%, (average of T1 seeds of 52 single copy T0 events), wheras VC-LMT593-1qcz rc had a Delta-4 DESATURASE conversion efficiency of 47% (average of T1 seeds of 241 single copy T0 events). This was not expected, and can be explained by transcript levels, which in turn determine protein levels. The transcript levels are affected by the genetic elements that flank the *Delta-4 DESATURASE* cassettes in VC-LMT593-1qcz rc. The observations between the two constructs is an unexpected finding and indicates that not only the genome but also the T-DNA itself impacts the Delta-4 DESATURASE conversion efficiency, that was dependant on "gene dosage" as described in Example 19. Furthermore, the data in Example 10 to 19 demonstrate that it was possible to insulate expression cassettes from such effects. As can be seen in those Examples 10-19 all single copy events were capable of producing almost exactly the same VLC-PUFA levels when eliminating all other sources that affect PUFA levels (e.g. different environments, plant-to-plant variability, seed oil content). This was particularly striking when comparing all the single copy events in Example 18. Comparing the total C20+C22 VLC-PUFA content, which was largely only controlled by how much was converted by the delta-12 desaturase and by the delta-6 desaturase, it was striking to observe there was virtually no difference between e.g. the single copy event LANPMZ obtained from the contruct combination VC-LJB2197-1qcz + VC-LLM337-1qcz rc, and all single copy events listed in Example 18. To this end, it is important to note that one side of the T-DNA that encodes either the entire pathway (Example 15 to 18) or at least the first steps of the pathway up to ARA and EPA production (Example 10 to 14) always contains the AHAS gene which confers herbicide tolerance but was not involved in the VLC-PUFA pathway. The other side of the T-DNA encodes either the entire pathway (Example 15 to 18) or at least the first steps of the pathway up to ARA and EPA production (Example 10 to 14) in most cases the *Delta-6 ELONGASE* from *Physcomitralla patens* (except in Example 13 and 14). As described in Example 19, the Delta-6 ELONGASE protein encoded by the *Physcomitrella patens* gene works close to maximum conversion efficiency (>90%), thus any increase in delta-6 elongase enzyme levels due to any effect that increases transcript levels will have virtually no effect on the C20 and C22 VLC-PUFA levels. Effectively, the T-DNA determining the total level of VLC-

PUFA accumulation are flanked on both sides by genes where expression level differences will have no impact on the VLC-PUFA accumulation. As these two genes were encoded by expression cassettes that were several thousand bp in size, it appears the genes inside the T-DNA were shielded/insulated from any effects the genomic environment could have on the expression level of those genes (e.g. the delta-12-desaturase, compare with Example 19). This effect was consistent with the observation that double copy events differ considerably more in total C20 and C22 VLC-PUFA levels: As in many cases the additional T-DNA insertions are not complete (see Example 10 to 18), resulting in exporsure of T-DNA internal genes are expoed to the genome. When these genes are susceptible to gene-dosage effects (the conversion efficiency of those genes depends on the amount of transcript and the derived amount of enzyme, compare with Example 19), then in some genomic locations the genomic envorinment boosted the transcript level.

Table 12: Definition of shorthands used for plant expression cassettes of this invention

Cassette shorthand	Promoter	Intron	Coding sequence	Terminator
USP/c-d6Elo(Pp_GA2)	p-VfUSP_684bp[LLL894]	i-Atss18_252[LJK36]	c-d6Elo(Pp_GA2)	t-CaMV35S
PXR/c-o3Des(Pir_GA)	p-LuPXR 1727bp[LLL823]	i-Atss15_758bp[LJK33]	c-o3Des(Pir_GA)	t-AtPXR 400bp[LLL823]
Conlinin/c-d5Des(Tc_GA2)	p-LuCnl(1064bp)	i-Atss14_377[LJK32]	c-d5Des(Tc_GA2)	t-AgrOCS 192bp[LED12]
SBP/c-d6Des(Ot_febit)	p-SBP	i-Atss2_455bp[LJK20]	c-d6Des(Ot_febit)	t-StCATHD-pA
PXR/c-d6Elo(Tp_GA2)	p-LuPXR 1727bp[LLL823]	i-Atss1_847bp[LJK19]	c-d6Elo(Tp_GA2)	t-AtPXR 400bp[LLL823]
Fae/c-d6Elo(Tp_GA2)	p-BnFae1	i-Atss1_847bp[LJK19]	c-d6Elo(Tp_GA2)	t-bnFae1
Napin/c-d12Des(Ps_GA)	p-Napin A/B	i-Atss14_377[LJK32]	c-d12Des(Ps_GA)	t-E9
USP/c-o3Des(Pi_GA2)	p-VfUSP_684bp[LLL894]	i-Atss18_252[LJK36]	c-o3Des(Pi_GA2)	t-CaMV35S
UBI/AHAS	p-PcUbi4-2[long]	part of PcUBI promoter	c-AtAHASL_A122T_S653N	t-AtAHAS-3'/UTR[ac321]
Conlinin/d4Des(Eg)	p-LuCnl(1064bp)	N/A	c-d4Des(Eg_GA)	t-AgrOCS 192bp[LED12]
SBP/d15Des(Ch)	p-VfSBP_perm3	N/A	c-d15Des(Ch_ERTp_GA)	t-StCATHD-pA
Conlinin/c-d4Des(Pl_GA)2_var1	p-LuCnl(1064bp)	i-Atss2_455bp[LJK20]	c-d4Des(Pl_GA)2	t-AgrOCS 192bp[LED12]
SETL/c-d5Des(Tc_GA2)	p-BnSETL-v1[1234bp]	N/A	c-d5Des(Tc_GA2)	t-BnSETL
SETL/c-o3Des(Pir_GA)	p-BnSETL-v1[1234bp]	N/A	c-o3Des(Pir_GA)	t-BnSETL
FAE/c-d5Elo(Ot_GA3)	p-BnFAE1	i-Atss1_847bp[LJK19]	c-d5Elo(Ot_GA3)	t-bnFAE1
SETL/c-o3Des(Pir_GA)	p-VfSBP_perm3	N/A	c-o3Des(Pir_GA)	t-StCATHD-pA
ARC/c-d4Des(Tc_GA)_var1	p-ARC5_perm1	N/A	c-d4Des(Tc_GA)	t-pvarc
ARC/c-d4Des(Tc_GA)_var2	p-ARC5_perm1	N/A	c-d4Des(Tc_GA3)	t-pvarc
Conlinin/c-d4Des(Pl_GA)2_var2	p-LuCnl(1064bp)	N/A	c-d4Des(Pl_GA)2	t-AgrOCS 192bp[LED12]
FAE/c-d4Des(Pl_GA)2	p-BnFAE1	i-Atss1_847bp[LJK19]	c-d4Des(Pl_GA)2	t-bnFAE1

Table 13: Order and orientation of plant expression cassettes on T-DNAs. Per column, expression cassettes are abbreviated according to Table 12 and listed from top to bottom in the following way: The top row indicated the right border of the first T-DNA introduced into the plant genome; the following proteins are all listed according to sense orientation of transcription, pointing away from the right border. The end of the first T-DNA was indicated by 'LB'. In case a second T-DNA was used to transfer all pathway genes into the plant genome according to example 7 to 11, the right border of this T-DNA was indicated as 'RB2'. The expression cassettes of the second T-DNA are listed in the following rows. Empty cells have been introduced to facilitate comparison of the different constructs.

VC-LJB2197-1qcz + VC-LLM306-1qcz rc	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2197-1qcz + VC-LLM338-3qcz rc	VC-LJB2755-2qcz + VC-LLM391-2qcz rc	VC-LJB2755-2qcz + VC-LLM217-1qcz rc	RTP10690-1qcz_F	RTP10691-2qcz	VC-LMT595-1qcz rc	VC-LMT593-1qcz rc
RB	RB	RB	RB	RB	RB	RB	RB	RB
USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)	USP/c-d6Elo(Pp_GA2)
Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)	Conlinin/c-d5Des(Tc_GA2)
SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)	SBP/c-d6Des(Ot_febit)
PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	FAE/c-d6Elo(Tp_GA2)	FAE/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)	PXR/c-d6Elo(Tp_GA2)
Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)	Napin/c-d12Des(Ps_GA)
			USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	PXR/c-o3Des(Pir_GA)	
UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS				
LB	LB	LB	LB	LB				
RB2	RB2	RB2	RB2	RB2				
Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)				Conlinin/c-d4Des(Pl_GA)2_var1	SETL/c-d5Des(Tc_GA2)	SETL/c-d5Des(Tc_GA2)	SETL/c-o3Des(Pir_GA)
FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	FAE/c-d5Elo(Ot_GA3)	USP/c-o3Des(Pi_GA2)
SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)							SETL/c-d5Des(Tc_GA2)
					ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2	ARC/c-d4Des(Tc_GA)_var2
USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)			USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	USP/c-o3Des(Pi_GA2)	PXR/c-o3Des(Pir_GA)
ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	ARC/c-d4Des(Tc_GA)_var1	SETL/c-d5Des(Tc_GA2)			Conlinin/c-d4Des(Pl_GA)2_var1
		Conlinin/d4Des(Eg)	Conlinin/d4Des(Eg)	Conlinin/c-d4Des(Pl_GA)2_var2		FAE/c-d4Des(Pl_GA)2	FAE/c-d4Des(Pl_GA)2	FAE/c-d5Elo(Ot_GA3)
					SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	SETL/c-o3Des(Pir_GA)	
					UBI/AHAS	UBI/AHAS	UBI/AHAS	UBI/AHAS

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VC-LJB2197-1qcz + VC-LLM306-1qcz rc	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2197-1qcz + VC-LLM338-3qcz rc	VC-LJB2755-2qcz + VC-LLM391-2qcz rc	VC-LJB2755-2qcz + VC-LLM217-1qcz rc	RTP10690-1qcz_F	RTP10691-2qcz	VC-LMT595-1qcz rc	VC-LMT593-1qcz rc
LB2	LB2	LB2	LB2	LB2	LB	LB	LB	LB

Example 5: Procedure for production of transgenic plants using a Co-Transformation approach

In general, the transgenic rapeseed plants were generated by a modified protocol according to DeBlock et al. 1989, Plant Physiology, 91:694-701). For the generation of rapeseed plants transgenic for two different T-DNAs, the binary vectors described in example 3 were transformed into *Agrobacterium rhizogenes* SHA001 (see WO2006024509 A2 for full description of the *Agrobacterium* used). For the transformation of rapeseed plants (cv. Kumily), a co-transformation strategy was used. Transformation was performed with two different agrobacteria strains harbouring one of the two different plasmids listed in Table 14 and described in detail in Example 3, Example 4, Example 6 and/or Example 7

Table 14: Overview of combinations used in Co-transformation Strategy described in Example 3 for generation of plants harboring two different T-DNAs

ID of Combination	Plasmid containing **T-DNA 1** harbored by *Agrobacterium tumefaciens* **clone** 1		Plasmid containing **T-DNA 2** harbored by *Agrobacterium tumefaciens* **clone 2**	
	Plasmid name	Selectable marker for transgenic plants	Plasmid name	Selectable marker for transgenic plants
A	VC-LJB2197-1qcz	AHAS	VC-LLM306-1qcz rc	None
B	VC-LJB2197-1qcz	AHAS	VC-LLM337-1qcz rc	None
C	VC-LJB2197-1qcz	AHAS	VC-LLM338-3qcz rc	None
D	VC-LJB2755-2qcz rc	AHAS	VC-LLM391-2qcz rc	None
E	VC-LJB2755-2qcz rc	AHAS	VC-LTM217-1qcz rc	None

Overnight cultures of the two strains intended to be co-transformed were prepared in YEB medium with antibiotics (20 mg/L chloramphenicol, 5 mg/L tetracycline, 25 mg/L Spectinomycin) and grown at 28°C. On the next day the optical density of the culture was checked at 600 nm wave length. It reached about 1.0. Cultures of lower optical density were extended in cultivation period. Cultures with an optical density of above 1.3 were diluted with YEB medium to an OD of approximately 0.2 and cultured until they reach an OD of 1.0.

Cultures were pelleted at about 4000 g and re-suspended in liquid MS medium (Murashige and Skoog 1962), pH 5.8, 3% sucrose with 100 mg/L Acetosyringone to reach an OD_{600nm} of 0.1.

The *Agrobacterium* suspensions corresponding to each of the two constructs to be co-transformed were mixed in equal parts and used for inoculation of hypocotyl segments prepared from 5 days old etiolated seedlings.

Germination took place on half concentrated MS medium, pH 5.6 – 5.8, 1% sucrose, at 23°C in the dark for 5 days. Hypocotyl segments of 4 to 7 mm length were inoculated by dipping. Infected explants were transferred to petri dishes with co-cultivation medium (MS medium, pH 5.6, 3% sucrose, 0.6 g/L MES (2-(N-Morpholino)ethanesulfonic acid), 18 g/L mannitol, 0.7% phytoagar (Duchefa Biochemie, PO Box 809 2003 RV Haarlem, Netherlands, part number SKU:P1003), 100 mg/L Acetosyringone, 200 mg/L L-Cysteine, 1 mg/L 2,4D (2,4-Dichlorophenoxyacetic acid)) carrying one layer of Whatman filter paper on its surface. Petri dishes were sealed with tape and incubated at 23 C under long day conditions (16 h light/8 h darkness) for three days. After the three days co-cultivation period explants were transferred to MS medium, pH 5.6, 3% sucrose, 0.6 g/L MES, 18 g/L mannitol, 07% Phytoagar, 1 mg/L 2,4D and 500 mg/L Carbenicillin to prevent *Agrobacterium* growth and incubated for a recovery period under the same physical conditions as for the co-cultivation for 7 days.

For selective regeneration explants were transferred after the recovery period to MS medium, pH 5.8, 3% sucrose, 0.7% Phytoagar, 2.5 mg/L $AgNO_3$, 3 mg/L BAP (6-Benzylaminopurine), 0.1 mg/L GA (Gibberellic acid), 0.1 mg/L NAA (1-Naphthaleneacetic acid), 500 mg/L Carbenicillin, 100 nM Imazethapyr (Pursuit) and cultured for two weeks under long day conditions as described above. Sub-cultivation takes place every two weeks. Hormones were stepwise reduced as follows: BAP 3 to 0.5 to 0.05 mg/L; GA (Gibberellic acid) 0.1 to 0.25 to 0.25 mg/L; NAA 0.1 to 0 to 0 mg/L.

Developing shootlets could be harvested after the second cycle of selective regeneration. Shootlets were cut and transferred to either Elongation/rooting medium (MS medium, pH 5.8, 2%sucrose, 100 mg/L myo-inositol, 40 mg/L Adenine sulphate, 500 mg/L MES, 0.4% Sigma Agar, 150 mg/L Timentin, 0.1 mg/L IBA (Indole-3-butyric acid)) or to rock wool/stone wool or foam mats (Grodan, GRODAN Group P.O. Box 1160, 6040 KD Roermond The Netherlands, or Oasis, 919 Marvin Street, Kent, OH 44240 USA) watered with 1/10 Vol. of MS medium, pH 5.8 without sucrose under ex vitro long day conditions in covered boxes.

Shoots were elongated and rooted in *in vitro* medium and were transferred directly to soil.

Either in vitro shoots or GH adapted shoots were sampled for molecular analysis.

The following modifications were successfully tested for transformation and were alternatively used to the above described protocol, once they had been worked out.

Seeds were germinated under low light conditions (< 50 µMol/m²s) using MSB5 medium from Duchefa (Duchefa Biochemie, PO Box 809 2003 RV Haarlem, Netherlands), pH 5.8, 3% sucrose and 0.8% Oxoid agar. Germination under light conditions produces explants, which are more stable and easier to handle compared to etiolated hypocotyls.

The inoculation method can vary but the method used in the invention was inoculating explants in a bath of *Agrobacterium* cells under gentle shaking up to 4 min and sieving the explants after the incubation with shaking. Under this condition the OD_{600nm} can be reduced up to 0.01.

Medium were used either autoclaved (except antibiotics, hormones, additives such as L-cysteine, Acetosyringon, imidazolinone components) or filter sterilized prepared (Agar component autoclaved, allowed to cool to 42 C and then used).

Example 6: Procedure for production of transgenic plants using BiBACs

For BiBAC transformation the same protocol as described for the co-transformation approach was used except that only one construct was used. According to the prokaryotic kanamycin resistance gene of binary plasmid 50 mg/L kanamycin was used instead of Spectinomycin for *Agrobacterium* growth. It was observed during the course of this work that *Agrobacterium* carrying BiBACs grow very slowly, often taking 18 hours to reach a liquid culture OD_{600nm} considered optimal for use in plant transformation.

The table below gives an example for some key data documented during the transformation of the construct LTM593

	VC-LTM593-1qcz rc
Explants inoculated	37 600
Shoots harvested	2 630
Shoots sampled and analyzed for gene AHAS	1 543
Transgenic events with gene AHAS	1 050
Transformation efficiency (%)	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	32.0

The amount of single copy events produced by the plant transformation protocol described above was 45% and 38% of vector backbone-free events selected after transformation of the constructs LTM593 and LTM595, respectively, were single copy events (see Table 15).

Table 15: **Statistics of single and double copy events with and without vector backbone in transformation experiments performed with the two BiBAC strains VC-LTM593-1qcz rc and VC-LTM595-1qcz rc**

	VC-LTM593-1qcz rc		VC-LTM595-1qcz rc	
	#	%	#	%
Number of transgenic events confirmed to contain at least 1 copy of gene c-AtAHAS	1050		217	
Single copy	535	50	92	42
Single copy, vector backbone-free	478	45	83	38
Double copy	320	30	49	23
Double copy vector backbone-free	227	22	41	18

One important key finding for successful transformation was the choice of *Agrobacterium* strain. While the original method (see De Block et al. (1989) Plant Physiology 91:694-701) used the *Agrobacterium tumefaciens* strain C58C1pMP90, the described method was based on the *Agrobacterium rhizogenes* strain SHA001 (see WO2006024509 A2 for SHA001 and SHA017). Even within *Agrobacterium rhizogenes* strains we have realized a clear response of transformation success to the strain and construct used (seeTable 16).

Table 16: **Impact of Agrobacterium rhizogenes strains on transformation success of BiBACs**

	VC-RTP10690-1qcz_f	VC-LTM593-1qcz rc
Strain used	**SHA017**	**SHA001**
Number of inoculated explants	60700	37600
Regeneration efficiency (%)	1.8	4.1
Shoots samples and analysed for gene AHAS	1084	1543
Number of transgenic plants based on the presence of gene c-AHAS	333	1050
Transformation efficiency (TE) (%)	0.6	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	69.3	32.0

Table 17: Transformation Efficiencies of the various plasmids and Agrobacterium strains used. With respect to the integration of the T-DNA, it was possible that multiple copies or single copies of intact or truncated or duplicated, or truncated and duplicated T-DNA's could be inserted into the genome. The terms copy or copies refer to the number of copies of a particular T-DNA or fragment of a T-DNA were inserted into the plant genome. The term locus refers to how many different locations within the plant genome the copy or copies of the T-DNA were inserted into. Locus is defined as region of disequilibrium within the genome an area which varies between plant species and even within cultivars of a given species. For the purpose of this definition this is within one genetic map unit or CentiMorgan.

Outcome	VC-LJB2197-1qcz + VC-LLM337-1qcz rc	VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc	VC-RTP10690-1qcz_f	VC-LTM593-1qcz rc
Agrobacterium strain	SHA001	SHA001	SHA017	SHA001
Transformation efficiency (TE) (%) based on the presence of gene AHAS	17	19.7	0.6	2.8
Percentage of events that grown on herbicide seelction plates but where confirmed using qPCR to lack the herbiced resitstance marker (%)	1.1	0.6	69.3	32.0
Genes of both T-DNAs present (% co-transformed)	11.2	15.1	n. a.	n. a.
% of single copy events **one locus integration**	0	0	100	100
Portion of selected "more copy events" (2 to 3 copies) with **one locus integration**	0	0	n.d.	25 from 33

Example 7: Seed Germination and Plant Growth in the Greenhouse and Field

Transformed plants were cultivated for seed production and phenotypic assessment in both the greenhouse and in the field. Greenhouse growth conditions were a sixteen hour light period followed by an eight hour dark period. The temperature was 20 degrees celsius during the light period (also called the day period) with a level of light corresponding to 200-300 micromoles of photons m-2 s-1 (this is the incident of light at the top of the plant and lights were adjusted in terms of distance from the plant to

achieve this rate). During the day period the range of light in the greenhouse varied between 130 and 500 micromoles of photons m-2 s-1. Getting out of the day range just cited triggered either the use of artificial light to bring the level up to 200-300 micromoles of photons m-2 s-1 or shading and/or shut off of lights to bring the level back to 200-300 micromoles of photons m-2 s-1. The dark period (also referred to as the night period) temperature was 18 C. Four hours before the light period began the temperature was lowered to 15 C for the remainder of the dark period. Plants were irrigated and treated for insects as necessary. The soil type was 50 % Floradur B Seed + 50 % Floradur B Cutting (including sand and perlite) provided by Floragard (Oldenburg, Germany). Plant growth was enhanced by nutrient supplementation. Nutrients were combined with the daily watering. A 0.1% (w/v) fertilizer solution (Hakaphos Blue 15(N) -10 (P) - 15(K), Compo GmbH & Co KG, Münster, Germany) was used to water the plants. Water was supplied on demand (e.g. depending on plant growth stage, water consumption etc.). To avoid cross-pollination, plants were bagged at the time when the first flowers opened. Plants were checked daily in order to ensure that all open flowers were covered by the bags. Open flowers that were not covered properly were removed.

For field grown plants, the plants were grown in six locations which correspond climatically to USDA growth zones 3a-4b and 5a, and five locations corresponding climatically to USDA growth zones 8a-9b and 11. The plants grown in the regions corresponding to USDA growth zones 3a-4b and 5a were grown in the summer and the plants grown in the regions corresponding to USDA growth zones 8a-9b and 11 were grown in the winter. Standard horticultural practices for canola were followed. Netting and other measures to protect from birds and insects were used as deemed necessary by the growers, as were herbicides and fertilizer applications. The planting density for all locations was eighty seeds per square meter with germination rate of 95 or better percent.

In the case where it was necessary to determine germination rates for the purpose of seed quality assurance or control, or where it was advantageous to germinate seeds to obtain cotyledons or seedling tissues, the following protocol was used:
150 mm by 15 mm petri-plates and Whatman (no. 2) filter paper cut into 120 mm disks were used. The filter paper was pre-moistened with sterile deionized water. One

hundred seeds of the appropriate line were obtained and spread evenly across the pre-moistened filter paper.

Clean and sterile tweezers were used to spread the seeds to obtain the uniform pattern as shown above. Additional sterile water was added to ensure the seeds and paper were wetted, but not floating (see above image). The total amount of water used per petri-plate was approximately 20 milliliters. Three plates were done for each genotype tested. The plates were sealed with surgical tape, VWR (1050 Satellite Blvd. Suwanee, GA 30024 USA) catalog number 56222-110. After the plates were sealed, they were then incubated in a germination chamber set to 90% humidity, set to a sixteen hour photoperiod with 20 degrees Celsius day temperature and 15 degrees Celsius night temperature. The light intensity was 90-120 micro-moles per square meter per second. Germination was scored twice, once at four days after placing the plates into the growth chamber and again at eight days after incubation.

Example 8: Lipid extraction and lipid analysis of plant oils

The results of genetic modifications in plants or on the production of a desired molecule, e.g. a certain fatty acid, were determined by growing the plant under suitable conditions, e.g. as described below, and analyzing the growth media and/or the cellular components for enhanced production of the desired molecule, e.g. lipids or a certain fatty acid. Lipids were extracted as described in the standard literature including Ullman, Encyclopedia of Industrial Chemistry, Bd. A2, S. 89-90 und S. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Bd. 17; Rehm et al. (1993) Biotechnology, Bd. 3, Kapitel III: "Product recovery and purification", S. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., und Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., und Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Bd. B3; Kapitel 11, S. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications.

It is acknowledged that extraction of lipids and fatty acids can be carried out using other protocols than those cited above, such as described in Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940, and Browse et al. (1986) Analytic Biochemistry 152:141-145. The protocols used for quantitative and qualitative analysis of lipids or fatty acids are described in Christie, William W., Advances in Lipid Methodology, Ayr/Scotland:

Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 S. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) u.d.T.: Progress in the Chemistry of Fats and Other Lipids CODEN.

To generate transgenic plants containing the genetic elements described in examples 3 and 4 for production of EPA and DHA in seeds, rapeseed (*Brassica napus*) was transformed as described in examples 5 and 6. Selected plants containing the genetic elements described in examples 3 and 4 were grown until development of mature seeds under the conditions cited in Example 7. Fatty acids from harvested seeds were extracted as described above and analyzed using gas chromatography as described above. The content (levels) of fatty acids is expressed throughout the present invention as percentage (weight of a particular fatty acid) of the (total weight of all fatty acids). Seed oil content is expressed throughout the present invention as percentage of (oil weight) of the (total oil weight of seeds).

Table 18: Fatty acids analyzed using gas chromatography

Systematic name	Trivial Name	Short hand 1	Short hand 2
Hexadecanoic acid	Palmitic acid	16:0	
(Z)-7-Hexadecenoic acid		16:1n-9	
(Z,Z,Z)-7,10,13-Hexadecatrienoic acid		16:3n-3	
Octadecanoic acid	Stearic acid	18:0	
(Z)-9-Octadecenoic acid	Oleic acid	18:1n-9	OA
(Z,Z)-9,12-Octadecadienoic acid	Linoleic acid	18:2n-6	LA
(Z,Z)-6,9-Octadecadienoic acid		18:2n-9	
(Z,Z,Z)-9,12,15-Octadecatrienoic acid	alpha-Linolenic acid	18:3n-3	ALA
(Z,Z,Z)-6,9,12-Octadecatrienoic acid	gamma-Linolenic acid	18:3n-6	GLA
(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid	Stearidonic acid	18:4n-3	SDA
Eicosanoic acid	Arachidic acid	20:0	
(Z)-11-Eicosenoic acid	Gondoic acid	20:1n-9	
(Z,Z)-11,14-Eicosadienoic acid		20:2n-6	
(Z,Z,Z)-11,14,17-Eicosatrienoic acid		20:3n-3	
(Z,Z,Z)-8,11,14-Eicosatrienoic acid	Dihomo-gamma-linolenic acid	20:3n-6	DHGLA
(Z,Z,Z,Z)-8,11,14,17-Eicosatetraenoic acid		20:4n-3	
(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid	Arachidonic acid	20:4n-6	ARA
(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid	Timnodonic acid	20:5n-3	EPA
Docosanoic acid	Behenic acid	22:0	
(Z)-13-Docosenoic acid	Erucic acid	22:1n-9	
(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic acid	Adrenic acid	22:4n-6	DTA
(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid	Clupanodonic acid	22:5n-3	DPAn-3
(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid	Osbond acid	22:5n-6	DPAn-6
(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid		22:6n-3	DHA

Example 9: Non-destructive analysis of lipids in single cotyledons of seedlings

Transformation of plants according to the methods described in Example 5 and Example 6 results in a random integration of the T-DNA into the genome. It was known that such integrations can also occur in a partial manner, furthermore multiple integrations of complete and partial T-DNAs can occur. A diploid plant contains one haploid chromosome set inherited from the ovule containing progenitor (for simplicity called the mother or female parent), and one haploid chromosome set inherited from the pollen donating progenitor (for simplicity called the father or male parent). In transformation of the T0 plant the T-DNA integrates into random position(s) of random chromosomes and will result in the maternal chromosome carrying the integration(s), and the corresponding paternal chromosome will not carry this integration, resulting in a seed heterozygous for the integration(s). Growing the T0 seed up results in a plant heterozygous for the T-DNA insert(s) and subsequent gametogenesis will result in both pollen and ovules that contain the T-DNA integration and in some pollen and ovules that do not carry the T-DNA insert(s), according to random segregation, as observed by Gregor Mendel (Mendel, 1866). Self pollination of the plant will

result in seeds which will be segregating for the T-DNA insertion(s) according to the ratios observed by Gregor Mendel (Mendel, 1866) and which are now part of the basic general knowledge in the life sciences. Due to the Mendelian segregation; for each integration of the T-DNA, one quarter (~25%) of the T1 seed have lost the integration. 50% of the T1 seed will carry the T-DNA integration either on the maternal chromosome (25%), or paternal chromosome (25%); these seeds are 'heterozygous' or 'hemizygous' related to the T-DNA integration. The remaining quarter (~25%) of the T1 seed will carry the T-DNA on the maternal and paternal chromosome; these seeds are 'homozygous' related to the T-DNA integration. For plants that follow such a sexual propagation, it is essential to genetically fix the T-DNA integration(s), by selecting progenies that are homozygous for the T-DNA integration(s); otherwise the T-DNAs insertion(s) and the trait conferred by the T-DNA insertion(s) will continue to segregate and might be lost over a number of generations. In order to identify T1 seedlings where each T-DNA integration that was essential for the trait was present, ideally homozygous, one can perform quantitative PCR to measure the copy number of the T-DNA integration(s) directly. Alternatively one can analyse the trait conferred by the presence of the T-DNAs, which at least enables the identification of all seeds that do not contain all T-DNA of interest (null-segregants). For all constructs described in Example 10 to Example 14, and where indicated, a non-destructive analysis of VLC-PUFA production was performed. To this end, T1 seeds were germinated in the dark for three days on wet filter paper. After three days, one of the two cotyledons was cut off to subject it to lipid analysis as described in Example 8: Lipid extraction and lipid analysis of plant oils, the other cotyledon, including the hypocotyl and root, was planted in soil. As an example, the result from the lipid content analysis of these cotyledons from segregating T1 seedlings of event LANPMZ obtained from the construct combination described in Example 11 are shown in Figure 22; the results of event LBDIHN obtained from the construct combination described in Example 15 are shown in Figure 23. In both of these figures, it is observed that one quarter of the seed produce a significant amount of VLC-PUFA, while producing wildtype levels of Oleic acid. One can furthermore see in both figures two additional clusters of seedlings, see Figure 23. Counting the number of seed in these respective clusters, a 1:2:1 segregation ratio was observed for the clusters that produce (~0 VLC-PUFA): (medium level of VLC-PUFAs): (high level of VLC-PUFAs). The observations demonstrate a relationship between 'gene dosage', that was the number of T-DNA copies present in the genome, and VLC-PUFA levels. For all constructs described in Example 10 to Example 13, and where indicated, this relationship was exploited to identify T1 plants where at least one T-DNA locus has become homozygous, or where multiple T-DNA integration loci are at least present, or some are homozygous while others still segregate. The applicability of this method can be demonstrated for event LANPMZ, see Figure 22, all T1 seeds of event LANPMZ are capable of producing EPA and DHA. As this requires the presence of both T-DNAs, it can be concluded that at least one copy of the T-DNA of VC-LJB2197-1qcz and one copy of the T-DNA of VC-LLM337-1qcz rc have inserted into the genome, likely at the same locus. 13 T1 seedlings of those 288 seedlings of event LANPMZ

having the highest VLC-PUFA levels have been selected and have been grown to mature plants. Copy number analysis on those 13 selected plants shown in Table 40 indicates that both T-DNAs are present in a single copy, and comparison of the T0 plant copy number results against the average result of the 13 T1 plants demonstrates that these single T-DNA insertions are homozygous (duplicated copy number). All results combined provide the information that the event LANPMZ contains the T-DNAs of construct VC-LJB2197-1qcz and the T-DNA of construct VC-LLM337-1qcz rc in one copy each, whereby both T-DNAs co-segregate in a single locus.

For a single T-DNA integration into the genome, 1 out of 4 T1 seed are expected to be homozygous for that T-DNA integration. For each additional T-DNA integration, just one quarter of all seed homozygous for all other T-DNA integrations are homozygous for the additional T-DNA integration, consequently for two T-DNA integration events into the genome 1 out of 16 T1 seed are expected to be homozygous for both T-DNA integration; for three T-DNA integration into the genome 1 out of 64 T1 seed are expected to be homozygous for all three T-DNA integration; for four T-DNA integration into the genome 1 out of 256 T1 seed are expected to be homozygous for all four T-DNA integration; and so forth. All plants Example 10 to Example 14 contain a minimum of two T-DNA insertion events (one from each plasmid) in order for the plant to contain all the necessary genes to generate all the required enzymes to reconstitute the PUFA pathway sufficiently to generate the VLC-PUFAs: DHA and EPA as well as ARA.

Example 10: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM306-1qcz rc (combination A in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc containing two different T-DNAs where cloned into agrobacteria, and plant tissue was incubated according Example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2197-1qcz or VC-LLM306-1qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained at least the T-DNA of VC-LJB2197-1qcz. Only those plants were kept, that also contained the T-DNA of plasmid VC-LLM306-1qcz rc as confirmed by PCR, conducted as described in Example 24, which contains a description of PCR used for gene expression and gene copy number determination. Only plants containing both the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM306-1qcz rc contain all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz, and the function of each of the elements, are listed in Table 1. The genetic elements of VC-LLM306-1qcz rc, and the function of each of the elements, are listed in Table 3. For convenience, all enzymes expressed in seeds of plants carrying

both T-DNA of VC-LJB2197-1qcz and VC-LLM306-1qcz rc that are required for EPA and DHA synthesis are additionally listed in Table 19.

Table 19: Combined list of useful genes of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc.

Genes encoding enzymes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Des(Ot_febit)	VC-LLM306-1qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LLM306-1qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LLM306-1qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	VC-LLM306-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM338-3qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM306-1qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during the summer.

The data in Table 20, Table 21 and Table 22 show that there was an increase in DHA and EPA content when comparing one versus two copies of each of the T-DNAs (VC-LJB2197-1qcz and VC-LLM306-1qcz rc) in the plant. The copy number of the construct VC-LJB2197-1qcz has been determined by measuring the left border of the T-DNA in the T0 generation

and not other genetic elements along the T-DNA (see Table 20). It was possible that the 88 plants representing the single copy category do in fact contain additional T-DNA insertions, and that the 86 plant representing the double copy category might in fact lack parts of the T-DNA. Therefore, due to insufficient data to correctly classify T0 plants into "single copy" and "double copy" groups, both populations overlap. Comparisons between two and three copies of the T-DNA's revealed that there was a minimal increase in DHA and EPA, suggesting that two copies of each gene was sufficient to reach maximum performance of the VLC-PUFA biosynthesis pathway (C20 and C22 PUFAs, including, but not limited to, EPA, DHA and ARA) when considering copy numbers up to three. Of note was that the majority of insertions in this example are one or two copy events. Table 23 indicates that with respect to the PUFA pathway or the copy number of the T-DNA encoding genes of the PUFA pathway, there was no significant affect on plant morphology or development when the plant carries one, two or three copies of the T-DNA's of interest.

Table 20: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d6Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
sc (n=88)	1.2										1.1	1.2		1.1
dc (n=86)	1.6										1.8	2.4		1.7
tc (n=5)	1.9										3.4	4.2		3.2

Table 21: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 20. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T0 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=88)	4.9 ± 0.9	0.3 ± 0.1	0.1 ± 0	2.9 ± 0.6	39.3 ± 8.2	28.4 ± 5.8	0.7 ± 4.4	5.8 ± 1.3	0.4 ± 0.6	0.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.6	0.3 ± 0.3	1.6 ± 0.8	0.9 ± 0.6	2.2 ± 1.7	3.3 ± 1.4	0.3 ± 0.1	0 ± 0	0.3 ± 0.3	1.3 ± 0.7	0 ± 0	0.5 ± 0.3	0.2 ± 0.3	
dc (n=86)	4.8 ± 1	0.3 ± 0.1	0.1 ± 0	3 ± 0.7	37.9 ± 9.2	27.9 ± 6.4	1.1 ± 5.7	5.8 ± 1.4	0.7 ± 0.7	0.1 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.8 ± 0.6	0.3 ± 0.3	1.6 ± 1	1 ± 0.5	2 ± 1.6	4 ± 2.1	0.3 ± 0.1	0 ± 0	0.6 ± 0.4	1.8 ± 0.9	0 ± 0	0.8 ± 0.5	0.4 ± 0.3	

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tc (n=5)	5 ± 0.9	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.7	39.1 ± 8.1	28.4 ± 4.5	0.5 ± 0.3	6.1 ± 1.5	0.9 ± 0.6	0.2 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.6	0.3 ± 0.2	2.1 ± 2.1	1.2 ± 1	1.9 ± 2.4	4 ± 2.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	1.9 ± 1.1	0 ± 0	0.8 ± 0.5	0.8 ± 0.9	

Table 22: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 20. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	4.9	0.2	0.0	3.5	23.1	34.2	0.2	5.4	0.4	0.2	0.8	0.9	2.5	1.1	3.0	2.4	3.5	13.5	0.3	0.0	0.0	0.0	0.0	0.0	0.0	
dc (n=1)	7.1	0.0	0.0	4.5	3.2	44.5	1.5	0.3	0.0	0.0	1.0	1.1	2.3	0.5	9.8	1.8	0.0	12.6	0.4	0.0	1.3	4.9	0.0	2.8	0.6	
tc (n=1)	3.4	0.1	0.0	2.8	29.4	27.4	0.6	6.6	1.0	0.3	0.7	0.9	1.1	0.5	1.5	1.6	2.6	10.4	0.3	0.0	1.1	5.1	0.0	1.9	0.5	

Table 23: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events were grouped into the categories indicated in the first column as described in Table 20. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T0 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=88)	68.6 ± 9.2	1 ± 0	1.5 ± 1.4	2.2 ± 1.7	3.1 ± 3.1	3 ± 0	3 ± 0	5 ± 0	3.3 ± 2.5	3.8 ± 0.6	115.9 ± 12.6	3.6 ± 0.4	4.1 ± 1.9
dc (n=86)	69.4 ± 8.8	1 ± 0	1.5 ± 1.1	2.2 ± 1.5	2.9 ± 2.7	3 ± 0	3 ± 0.2	5 ± 0	2.9 ± 2.3	3.8 ± 0.6	115.8 ± 10.2	3.7 ± 0.4	4.5 ± 1.8
tc (n=5)	72.2 ± 8.2	1 ± 0	1 ± 0	1.6 ± 1.3	2.4 ± 0.9	3 ± 0	3 ± 0	5 ± 0	2.4 ± 1.5	4 ± 0	117 ± 9.7	4 ± 0.5	4 ± 0.8

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during winter

The copy number analysis indicates that LALTHK was homozygous for two copies of the VC-LJB2197-1qcz T-DNA and homozygous for one copy of the VC-LLM306-1qcz rc while LALJCX was homozygous for two copies of both T-DNAs (VC-LJB2197-1qcz and VC-LLM306-1qcz rc). The other events were still partially segregating for the T-DNAs but contained at least one copy of each T-DNA in all plants. Event LALTHK had no accumulation of DHA, which illustrates the effects of truncations which can occur during insertion of the T-DNA. The events, with the exception of LALTHK, are, within error, similar to one another in terms of EPA+DHA accumulation. The similarity in copy numbers of the events, see Table 23, indicates that insertion site effects that could enhance or repress gene expression are impacting all events equally. The lack of significant variation in EPA and DHA accumulation suggests that there may be a buffering effect in the construct design, such that the T-DNA's integrate into the genome in a manner that minimizes positional effects in gene expression in the T-DNA. The event with the highest VLC-PUFA accumulation, in particular EPA and DHA, was LALFWA which had a maximum accumulation of 4.2 percent DHA and 16 percent EPA with respect to total fatty acid content in the mature seed, but on the average was similar to the other events.

Table 24: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or on case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d6Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALJAU (n=18)	3.1 (T0: 2)									1.3	(T0: 1.1)			
LALFWA (n=16)	3.1 (T0: 1.8)									2.6	(T0: 1.9)			(T0: 1.9)
LALJDF (n=17)	3.2 (T0: 2)									3.3	(T0: 2)			
LALTHK (n=15)	3.8 (T0: 2.1)									2.1	(T0: 1.1)			
LALTLE (n=18)	3.4 (T0: 2.8)									2.4	(T0: 2.4)			
LALIVY (n=16)	3 (T0: 1.9)									3.1	(T0: 2.1)			
LALJCX (n=16)	3.4 (T0: 1.9)									3.4	(T0: 1.8)			

Table 25: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b

LALJAU (n=18)	4.5 ± 0.1	0.1 ± 0	0 ± 0	3.6 ± 0.4	21.5 ± 1.8	31.6 ± 0.9	0.8 ± 0.2	3.3 ± 0.3	1.9 ± 0.7	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.8 ± 0.4	0.1 ± 0.2	3.7 ± 0.6	1.6 ± 0.4	8.6 ± 2.7	9.6 ± 1.4	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.3 ± 0.6	0 ± 0	1.7 ± 0.5	0.1 ± 0.2	
LALFWA (n=16)	4.5 ± 0.5	0.1 ± 0	0 ± 0	3.5 ± 0.5	20 ± 2	30 ± 2.1	1 ± 0.1	3.8 ± 0.4	2.6 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0	0.2 ± 0.1	0 ± 0	3.7 ± 0.4	1.9 ± 0.4	5.6 ± 2.3	11.6 ± 2.2	0.2 ± 0.1	0 ± 0	1.4 ± 0.3	4.7 ± 1.1	0 ± 0	2.6 ± 0.8	0.5 ± 0.4	0.4 ± 0
LALJDF (n=17)	4.3 ± 0.7	0.2 ± 0.1	0 ± 0.1	3.3 ± 0.5	20.7 ± 4.1	29.3 ± 4	2.5 ± 5.9	4.1 ± 0.4	2.4 ± 0.5	0.7 ± 1.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0 ± 0	2.7 ± 0.4	1.7 ± 0.3	4.6 ± 0.6	13.1 ± 3	0.3 ± 0	0 ± 0	0.9 ± 0.3	4.7 ± 1.2	0.1 ± 0.3	2.2 ± 0.6	0.2 ± 0.2	0.2 ± 0
LALTHK (n=15)	4.7 ± 0.6	0.1 ± 0.1	0 ± 0	3.7 ± 0.4	21.4 ± 1.8	32.9 ± 1.9	0 ± 0.1	4.9 ± 0.6	0.6 ± 0.1	0 ± 0	1 ± 0.1	0.9 ± 0.1	1.7 ± 0.3	0.7 ± 0.2	2.2 ± 0.3	1.6 ± 0.2	4.3 ± 0.8	18.9 ± 2.5	0.4 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	
LALTLE (n=18)	4.6 ± 0.6	0.1 ± 0	0 ± 0	3.6 ± 0.4	24.1 ± 4.5	29.8 ± 2.2	1 ± 0.3	4.2 ± 0.5	2 ± 0.6	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0 ± 0.1	3 ± 0.2	2 ± 0.3	3.8 ± 0.9	10.9 ± 3	0.1 ± 0.1	0 ± 0	1.4 ± 0.6	4.7 ± 1.1	0 ± 0	2.3 ± 0.8	0.2 ± 0.3	0.6 ± 0.1
LALIVY (n=16)	5.4 ± 1	0.2 ± 0.1	0 ± 0	3.6 ± 0.4	21.6 ± 2.9	30.9 ± 2	0.9 ± 0.2	4.4 ± 0.5	2.1 ± 0.5	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	2.8 ± 0.2	1.9 ± 0.3	4.2 ± 1.2	10.4 ± 1.8	0.3 ± 0.1	0 ± 0	1.3 ± 0.3	4.4 ± 0.9	0 ± 0	2.4 ± 0.6	0.6 ± 0.2	0.5 ± 0.3
LALJCX (n=16)	5 ± 0.1	0.2 ± 0	0 ± 0	3.4 ± 0.2	23.9 ± 1.6	32.4 ± 1.1	1.1 ± 0.1	4.1 ± 0.4	2.5 ± 0.3	0.4 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0	0 ± 0	2.5 ± 0.7	1.3 ± 0.3	4.2 ± 0.3	9.9 ± 0.7	0.3 ± 0	0 ± 0	1.1 ± 0.2	3.8 ± 0.3	0 ± 0	1.7 ± 0.2	0.4 ± 0.2	

Table 26: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
LALJAU (n=1)	4.9	0.2	0.0	4.7	18.8	30.6	1.1	3.1	3.6	0.4	1.0	0.6	0.1	0.0	2.4	1.1	5.7	12.6	0.4	0.0	1.3	4.4	0.0	2.8	0.0	0.0
LALFWA (n=1)	3.8	0.1	0.0	3.2	16.1	26.2	1.2	4.4	3.0	0.5	0.7	0.7	0.3	0.1	2.9	2.4	3.6	16.0	0.1	0.0	1.9	7.4	0.0	4.2	0.7	0.4
LALJDF (n=1)	3.5	0.1	0.1	2.8	15.8	27.7	1.2	3.7	3.6	0.5	0.7	0.7	0.3	0.0	2.6	1.9	5.5	18.0	0.3	0.0	1.4	6.2	0.0	3.2	0.1	0.2
LALTHK (n=1)	4.7	0.0	0.0	4.2	18.6	30.7	0.0	4.6	0.6	0.0	1.1	0.9	2.0	1.0	2.2	1.7	4.6	22.7	0.4	0.0	0.0	0.0	0.0	0.0	0.0	
LALTLE (n=1)	3.3	0.2	0.1	2.8	15.0	24.9	1.5	3.3	3.3	0.5	0.7	0.6	0.5	0.0	3.1	2.2	5.7	17.6	0.3	0.0	2.4	6.9	0.0	4.1	0.5	0.5
LALIVY (n=1)	5.9	0.3	0.0	3.9	16.5	26.5	0.9	4.2	2.3	0.5	0.9	0.7	0.5	0.3	2.6	2.2	3.9	14.2	0.4	0.0	1.7	6.7	0.0	4.2	0.6	0.0
LALJCX (n=1)	6.1	0.7	0.2	2.3	22.0	15.1	15.3	6.6	2.5	0.5	0.6	0.6	0.5	0.0	1.2	0.9	4.6	10.0	0.4	0.0	0.8	5.5	0.0	3.5	0.0	0.0

Table 27: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique

(9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad),

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
LALJAU (n=18)	62.2 ± 2.3	1 ± 0	1 ± 0	1 ± 0	1.9 ± 1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.7	4 ± 0	129.7 ± 5.5	4.7 ± 0.2	2.1 ± 1
LALFWA (n=16)	61.6 ± 3.3	1 ± 0	1.2 ± 0.8	1 ± 0	3.5 ± 2.4	3 ± 0	3 ± 0	5 ± 0	3.1 ± 2.6	3.9 ± 0.5	127.7 ± 6.2	4.9 ± 0.4	3.5 ± 1.8
LALJDF (n=17)	58.7 ± 2.6	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	128 ± 6.2	5 ± 0.2	2.4 ± 0.6
LALTHK (n=15)	62.1 ± 5.5	1 ± 0	1 ± 0	1 ± 0	1.7 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	124.3 ± 5	4.8 ± 0.3	3.1 ± 1.2
LALTLE (n=18)	67.8 ± 6.7	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.7 ± 5.2	4.4 ± 0.3	4.2 ± 1.7
LALIVY (n=16)	69.5 ± 6.2	1 ± 0	2.7 ± 1.4	1.3 ± 0.6	2.4 ± 1.4	3 ± 0	3 ± 0	5 ± 0	1.6 ± 0.9	3.3 ± 1	127.1 ± 5.8	4.4 ± 0.4	4.9 ± 3.2
LALJCX (n=16)	64.2 ± 2.7	1 ± 0	2.7 ± 0.7	1 ± 0	1.4 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	131.4 ± 5.7	4.5 ± 0.3	2.2 ± 0.9

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Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in greenhouses during summer

Copy number analysis, see Table 28, indicates that the chosen events are largely homozygous for the genes encoded on the T-DNAs of VC-LJB2197-1qcz and VC-LLM306-1qcz rc. All constructs but LALTLE had two copies of the T-DNAs inserted into the genome. LALTLE appeared to have more than two copies with some segregation still ongoing. Based on the copy number analysis, LALJDF had integrated one copy of the d4Des(Eg_GA) gene and LALTLE was segregating for one or two copies of that gene. The EPA and DHA data in Table 29 and Table 30 indicated the events perform equally well with perhaps LALJDF accumulating less DHA, based on Table 30. Plant morphology was the same for all the events examined. As discussed in part B, the event to event variation was minimal, suggesting that the impacts of insertion site position effects (both negative and positive) were similar for all events. The lack of insertion site effects indicates that the plasmid design/T-DNA topology was exerting a normalizing effect/mitigates insertion site effects.

Table 28: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T2 plants that where measured per event. The T2 plants underwent a selection from 250 segregating T2 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or in case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T2 plants carry a heterozygous locus.

	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Des(Ot_febit) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM306-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-p-BnFAE_t-PvARC located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
Event	c-AHA5	j-t-E9-p3-2	c-d6Elo (Tp_GA)	j-t-StCAT_p2_p-LuPXR	c-d6Des(Ot_febit)	j-t-CaMV_p-LuCnl-2	j-i-Atss18_c-d5Elo(Pp_GA2)	c-d6Des(Ot_febit)	j-p-BnFAE_t-PvARC	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALFWA (n=30)	4.0	3.6	3.7	3.9	7.7	4.6	3.7	7.7	3.5	3.7			3.5	3.5
LALJDF (n=30)	4.2	3.9	3.9	3.8	8.5	3.3	3.5	8.5	5.1	4.1			4.0	2.2
LALTLE (n=30)	5.2	4.6	4.7	5.0	8.9	4.4	1.8	8.9	2.6	3.6			4.6	3.2
LALIVY (n=30)	4.1	3.6	3.7	3.8	8.1	4.4	5.4	8.1	3.7	4.0			3.8	3.5

Table 29: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc The events are indicated in the first column, along with the number of T3 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b

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LALFWA (n=30)	4.9 ± 0.2	0.3 ± 0.1	0.2 ± 0	4.4 ± 0.2	19.9 ± 0.9	29.5 ± 0.8	1.3 ± 0.1	3.2 ± 0.2	3 ± 0.2	0.4 ± 0	0.9 ± 0.1	0.6 ± 0	0.3 ± 0	0 ± 0	3.8 ± 0.6	2.1 ± 0.3	4 ± 0.4	11.3 ± 0.6	0.3 ± 0	0 ± 0	1.7 ± 0.2	4.3 ± 0.3	0 ± 0	2.5 ± 0.2	0.7 ± 0.2	0.3 ± 0
LALJDF (n=30)	5.4 ± 0.3	0.3 ± 0	0.2 ± 0	4.4 ± 0.2	21.4 ± 0.7	30.1 ± 1.1	1.2 ± 0.1	3.8 ± 0.3	2.7 ± 0.2	0.4 ± 0	0.9 ± 0	0.6 ± 0	0.1 ± 0.1	0 ± 0	2.2 ± 0.2	1.4 ± 0.1	4.3 ± 0.2	12.4 ± 0.7	0.2 ± 0.1	0 ± 0	1 ± 0.1	4.4 ± 0.4	0 ± 0	2.2 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
LALTLE (n=30)	5.2 ± 0.4	0.3 ± 0	0.2 ± 0.1	4.6 ± 0.3	20.3 ± 0.8	28.7 ± 0.8	1.3 ± 0.2	3.4 ± 0.3	2.8 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.5 ± 0.1	0.3 ± 0.1	0.1 ± 0.1	2.3 ± 0.2	1.6 ± 0.2	3.9 ± 0.2	12.1 ± 0.7	0.2 ± 0.1	0 ± 0	1.9 ± 0.2	4.9 ± 0.3	0 ± 0	2.9 ± 0.4	0.5 ± 0.1	0.3 ± 0.1
LALIVY (n=30)	5.3 ± 0.6	0.3 ± 0.1	0.2 ± 0	4.8 ± 0.5	20.4 ± 1.4	29.6 ± 1	1.2 ± 0.2	3.6 ± 0.2	2.7 ± 0.3	0.4 ± 0	0.9 ± 0.1	0.6 ± 0	0.4 ± 0.1	0.1 ± 0.1	2.5 ± 0.3	1.7 ± 0.3	4 ± 0.7	11.4 ± 0.7	0.3 ± 0	0 ± 0	1.5 ± 0.1	4.5 ± 0.4	0 ± 0	2.7 ± 0.4	0.5 ± 0.2	0.3 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 30: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LALFWA (n=1)	5.4	0.4	0.2	4.6	17.5	27.9	1.1	2.9	3.3	0.4	0.9	0.5	0.3	0.0	3.7	2.0	4.5	12.7	0.3	0.0	2.2	5.0	0.0	3.2	0.6	0.3
LALJDF (n=1)	5.1	0.3	0.2	4.5	19.7	28.9	1.1	3.8	2.8	0.5	0.9	0.6	0.1	0.0	2.4	1.6	4.4	13.7	0.3	0.0	1.1	5.1	0.0	2.5	0.2	0.2
LALTLE (n=1)	5.8	0.1	0.1	5.5	20.1	27.3	1.9	3.1	3.5	0.2	0.4	0.3	0.2	0.0	1.9	0.7	4.0	13.4	0.1	0.0	1.1	5.5	0.0	4.2	0.2	0.1
LALIVY (n=1)	5.5	0.4	0.2	4.4	18.4	28.6	1.1	3.4	3.3	0.4	0.9	0.5	0.3	0.0	2.2	1.4	5.3	12.8	0.3	0.0	1.8	5.0	0.0	3.4	0.2	0.2
LALHCY (n=1)	7.6	1.1	0.7	2.3	19.7	25.6	0.9	6.3	3.6	0.7	0.8	0.6	0.0	0.0	1.2	1.1	3.9	10.4	0.0	0.0	1.0	7.0	0.0	3.4	0.0	0.0

Table 31: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes(#), PH: plant height (cm), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	Oil	Protein

LALFWA (n=30)	57.3 ± 2.2	9 ± 0	8.9 ± 0.4	8.9 ± 0.4	7.2 ± 1	3 ± 0	3 ± 0	5 ± 0	8 ± 1.2	3.9 ± 0.4	126.8 ± 4.3		
LALJDF (n=30)	58.1 ± 2.5	9 ± 0	9 ± 0	9 ± 0	7.7 ± 0.5	3 ± 0	3 ± 0	5 ± 0	8.2 ± 0.9	4 ± 0	130.2 ± 0.9		
LALTLE (n=30)	59.9 ± 2.8	9 ± 0	8.1 ± 1.3	8.8 ± 0.5	7.2 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.7 ± 1.3	3.9 ± 0.5	128 ± 2.8		
LALIVY (n=30)	59.6 ± 2.9	9 ± 0	8.8 ± 0.9	8.6 ± 1	6.8 ± 1.3	3 ± 0	3 ± 0	5 ± 0	7.3 ± 1.7	3.8 ± 0.6	126.8 ± 3.3		
WT Kumily (n=46)												34.9 ± 1.1	32.2 ± 1

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

The field data on Table 32 and Table 33 indicate a consistent performance across generations for T2 and T3. The data show that the PUFA (EPA and DHA) accumulation was higher for greenhouse grown plants. Besides levels in VLC-PUFA, there was also a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 34 and Table 31). Results of this analysis are described in Example 20. The field data also demonstrate that the greenhouse data accurately indicated the consistency between events with respect to EPA and DHA accumulation, though not overall levels. As remarked upon in part C, the event to event variation was low for this construct, indicating that the T-DNAs/T-DNA design seem to be exerting a buffering/mitigating effect on gene silencing and other positional effects.

Table 32: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field corresponding to USDA growth zones 3a-4b and 5a. Field trials of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc are given below. The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LALFWA (n=30)	5.1 ± 0.2	0.3 ± 0.1	0.1 ± 0	2.6 ± 0.2	25.1 ± 1.8	30.8 ± 1.4	1.1 ± 0.1	5.9 ± 0.9	1.8 ± 0.2	0.3 ± 0.1	0.7 ± 0	0.7 ± 0	0.4 ± 0.1	0.1 ± 0.1	2.6 ± 0.3	1.4 ± 0.2	4.4 ± 1.2	8.5 ± 1.2	0.3 ± 0	0 ± 0	1.2 ± 0.2	4.4 ± 0.5	0 ± 0	1.4 ± 0.2	0.5 ± 0.2	0.3 ± 0.1
LALJDF (n=30)	5.1 ± 0.3	0.3 ± 0	0.1 ± 0	2.7 ± 0.2	24.4 ± 1.7	29.4 ± 1.5	1.2 ± 0.1	5.6 ± 0.4	1.8 ± 0.2	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.4 ± 0.1	0.1 ± 0	2.2 ± 0.1	1.3 ± 0.1	4.5 ± 0.4	11.1 ± 0.9	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	5.1 ± 0.4	0 ± 0	1.4 ± 0.2	0.1 ± 0.1	0.4 ± 0.1
LALTLE (n=30)	5.4 ± 0.2	0.3 ± 0.1	0.1 ± 0	2.7 ± 0.2	26.4 ± 3.2	28.1 ± 1	1.2 ± 0.2	5.6 ± 0.6	1.7 ± 0.3	0.3 ± 0.1	0.7 ± 0	0.7 ± 0	0.5 ± 0.2	0 ± 0.1	2.1 ± 0.2	1.2 ± 0.1	3.9 ± 0.5	9.6 ± 1.2	0.2 ± 0.1	0 ± 0	1.5 ± 0.2	5.3 ± 0.6	0 ± 0	1.5 ± 0.2	0.4 ± 0.2	0.5 ± 0.1
LALIVY (n=29)	5.2 ± 0.4	0.3 ± 0.1	0.1 ± 0	2.9 ± 0.3	26.7 ± 3.1	28.3 ± 2.2	1 ± 0.2	6.3 ± 0.6	1.4 ± 0.3	0.3 ± 0	0.7 ± 0.1	0.8 ± 0.1	0.6 ± 0.2	0.2 ± 0.1	2.1 ± 0.2	1.4 ± 0.1	3.3 ± 0.5	9.2 ± 1.1	0.3 ± 0.1	0 ± 0	1.3 ± 0.1	5 ± 0.5	0 ± 0	1.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ± 4.2	23.3 ± 1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

Table 33: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a field trials of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
LALFWA (n=1)	5.2	0.3	0.1	2.6	25.0	28.2	1.1	5.0	2.1	0.4	0.6	0.5	0.3	0.0	2.7	1.4	4.5	10.5	0.3	0.0	1.4	5.2	0.0	1.8	0.4	0.3
LALJDF (n=1)	5.7	0.3	0.1	2.7	23.1	23.6	1.3	6.0	2.3	0.4	0.8	0.8	0.4	0.0	2.3	1.3	5.4	13.5	0.3	0.0	1.0	6.3	0.0	1.8	0.0	0.3
LALTLE (n=1)	5.5	0.3	0.1	2.6	24.9	26.7	1.3	4.7	2.1	0.4	0.7	0.6	0.4	0.1	2.1	1.2	5.0	10.9	0.3	0.0	1.8	5.8	0.0	1.9	0.2	0.5
LALIVY (n=1)	5.9	0.2	0.1	3.5	24.5	23.7	1.3	5.6	2.1	0.4	0.9	0.8	0.5	0.0	2.5	1.4	4.8	11.3	0.3	0.0	1.7	5.9	0.0	1.9	0.4	0.4

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Table 34: Phenotypic rating of T2 plants cultivated in the field of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM306-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LALFWA (n=30)	38.2 ± 1	27.9 ± 0.6
LALJDF (n=30)	37.6 ± 1.2	27.9 ± 0.8
LALTLE (n=30)	38.2 ± 0.9	27.5 ± 0.7
LALIVY (n=29)	37.6 ± 0.6	27.8 ± 0.5
WT Kumily (n=60)	38.7 ± 1.1	

Example 11: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM337-1qcz rc (combination B in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc containing two different T-DNAs were cloned into agrobacteria, and plant tissue was incubated according to Example 5 at the same time with these two agrobacterial cultures that were identical apart from containing either VC-LJB2197-1qcz or VC-LLM337-1qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2197-1qcz. Only those plants were kept, that also contained the T-DNA of plasmid VC-LLM337-1qcz rc as confirmed by PCR, conducted as described in Example 24, which contains PCR protocols for both gene expression and copy number analysis. Only plants containing the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM337-1qcz rc combined all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz and the function of each element were listed in Table 1. The genetic elements of VC-LLM337-1qcz rc and the function of each element were listed in Table 4. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2197-1qcz and VC-LLM337-1qcz rc that were required for EPA and DHA synthesis are additionally listed on Table 35.

Table 35: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length	Enzymatic function and source of encoded protein
c-o3Des(Pi_GA2)	VC-LLM337-1qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LLM337-1qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	VC-LLM337-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM337-1qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM337-1qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during summer

Table 34 indicates that the T-DNA integrated as predominantly single and double copies. As observed in Example 10 there was an increase in EPA and DHA between one copy and two copies of the T-DNA from the two constructs, see Table 36, but less of a difference between two and three copies. The T1 data in Table 37 reflects this as well. As noted in Example 10, there was no observed alteration of the phenotype of the plants bearing the T-DNA from both constructs, regardless of copy number (up to three).

Table 36: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Category of T0 plants	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.							Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.							
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
sc (n=563)	1.1					1.1			1.2	1.2	1.2	1.2			1.2
dc (n=423)	1.4					1.6			2.0	1.9	2.0	1.8			2.2
tc (n=17)	2.0					2.6			2.8	1.8	3.2	2.4			3.8

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Table 37: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=563)	5.1 ± 0.6	0.3 ± 0.1	0.1 ± 0	3 ± 0.5	40.6 ± 6.2	29.4 ± 4.2	0.7 ± 4.7	6 ± 1.1	0.5 ± 0.6	0.1 ± 0.2	0.8 ± 0.1	0.9 ± 0.2	0.9 ± 0.6	0.3 ± 0.3	1.6 ± 0.8	0.9 ± 0.5	2.1 ± 1.3	3.4 ± 1.2	0.3 ± 0.1	0 ± 0	0.4 ± 0.3	1.5 ± 0.5	0 ± 0	0.6 ± 0.3	0.3 ± 0.3	
dc (n=423)	5 ± 0.8	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.6	38.6 ± 8.2	28.8 ± 5.3	1.1 ± 5.9	5.9 ± 1.2	0.7 ± 0.6	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	0.8 ± 0.6	0.3 ± 0.3	1.7 ± 0.9	1 ± 0.5	2.1 ± 1.5	4.3 ± 2	0.3 ± 0.1	0 ± 0	0.6 ± 0.4	1.9 ± 0.9	0 ± 0	0.8 ± 0.5	0.4 ± 0.3	
tc (n=17)	5 ± 0.9	0.3 ± 0.1	0.1 ± 0	3.1 ± 0.7	39.1 ± 8.1	28.4 ± 4.5	0.5 ± 0.3	6.1 ± 1.5	0.9 ± 0.6	0.2 ± 0.1	0.8 ± 0.2	0.9 ± 0.2	0.7 ± 0.6	0.3 ± 0.2	2.1 ± 2.1	1.2 ± 1	1.9 ± 2.4	4 ± 2.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	1.9 ± 1.1	0 ± 0	0.8 ± 0.5	0.8 ± 0.9	

Table 38: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	3.6	0.1	0.1	2.8	37.6	22.1	0.8	6.4	0.5	0.3	0.8	1.2	1.1	0.6	1.8	2.1	2.9	9.0	0.4	0.0	0.5	3.5	0.0	1.6	0.3	
dc (n=1)	7.1	0.0	0.0	4.5	3.2	1.5	44.5	0.3	0.0	0.0	1.0	1.1	2.3	0.5	9.8	1.8	0.0	12.6	0.4	0.0	1.3	4.9	0.0	2.8	0.6	
tc (n=1)	3.4	0.1	0.0	2.8	29.4	27.4	0.6	6.6	1.0	0.3	0.7	0.9	1.1	0.5	1.5	1.6	2.6	10.4	0.3	0.0	1.1	5.1	0.0	1.9	0.5	

Table 39: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 36. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed,

1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=563)	66.1 ± 8.3	1 ± 0	1.4 ± 1.1	1.7 ± 1.6	3 ± 2.8	3 ± 0	3 ± 0.1	5 ± 0	3.2 ± 2.6	3.8 ± 0.6	119.5 ± 9.8	4.7 ± 0.6	4.8 ± 2.5
dc (n=423)	66 ± 7.5	1 ± 0	1.4 ± 1.2	1.7 ± 1.4	3 ± 2.9	3 ± 0	3 ± 0.1	5 ± 0.1	3.1 ± 2.6	3.7 ± 0.7	119.2 ± 9.5	4.6 ± 0.6	5 ± 2.3
tc (n=17)	64.6 ± 7.4	1 ± 0	1.5 ± 1.5	2.8 ± 2.5	4.4 ± 3.7	3 ± 0	3 ± 0	5 ± 0	4.5 ± 3.5	3.9 ± 0.5	117.1 ± 10.5	4.6 ± 0.4	6.5 ± 2.2

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during winter

The data on Table 39 indicate that the integration of these two T-DNA's (VC-LJB2197-1qcz and VC-LLM337-1qcz rc), has occurred in such a way as to introduce copy number variation of individual genes on a given T-DNA (indicating truncations and deletions along with multiple copies being inserted). For example the event LAMABL on Table 39 was segregating for a single copy of AHAS (homozygous), two copies of j-t-StCAT_p2_p-LuPXR (homozygous), possibly three copies of c-d6Elo(Pp_GA) likely homozygous, though it could be three copies which are not homozygous for all three, and three copies of j-i-Atss18_c-d6Elo(Pp_GA2) (homozygous for all three). Data on Table 42 to Table 45 for fatty acid profile indicates some variation among the events, though not large differences. The highest event average for both DHA and EPA for the events listed on Table 41 was LAMRHL which has DHA of 1.9 and EPA of 10.5 with respect to percent of the total fatty acid content of the seed and contains what was likely a single copy of the T-DNA of of VC-LJB2197-1qcz still segregating, while VC-LLM337-1qcz rc seems to be a single copy homozygous insertion. The event, LANMGC, with the lowest levels of EPA and DHA combined, contained EPA of 3.7 and 0.8 for DHA with respect to percent of the total fatty acid content of the seed. LANMGC appeared to be homozygous single copy for VC-LJB2197 and carried at least two separate integrations of VC-LLM337. For the highest single plant level of EPA and DHA, event LAMRHL had 5 percent of DHA and 13.7 percent of EPA with respect to percentage of total fatty acids in the seed, Table 43. The data indicate that the location of the insertion site is important for EPA and DHA accumulation in this combination of constructs. As seen in previous examples, comparison of single copy insertions versus double copy insertions revealed that between single copy and double copy containing plants there was an increase in VLC-PUFA levels, but between double and triple copy containing plants there was less distinction. Table 46 displays phenotypic scoring/assessment and shows some small differences in aerial phenotype among events and between the transformed plants and untransformed reference.

Table 40: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or in the case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LALHCY (n=15)	2.9 (T0: 1.8)	4.4		4.6	3.5	1.9 (T0: 1.1)	2.0	2.9	2.9 (T0: 2.1)		(T0: 2.6)	(T0: 2.1)	4.5		3.0
LALIAO (n=15)	3 (T0: 1.8)	2.7		3.3	3.6	2.8 (T0: 1.9)	3.1	3.2	2.8 (T0: 2)		(T0: 2.2)	(T0: 1.9)	2.9		1.5
LALJKA (n=15)	2 (T0: 1.1)	2.0		2.0	4.1	3.6 (T0: 1.9)	3.8	3.5	3.8 (T0: 2)		(T0: 1.9)		4.0		5.5
LALLTL (n=11)	1.7 (T0: 1.8)	3.5		3.5	3.1	3.2 (T0: 3)	3.2	2.3	2.4 (T0: 2)		(T0: 1.9)		2.3		2.8
LALQAM (n=15)	3.4 (T0: 1.9)	3.3		3.3	3.1	3.3 (T0: 1.9)	3.3	2.9	3.1 (T0: 2)		(T0: 2.1)		1.6		1.6
LALQDS (n=14)	2.7 (T0: 2)	2.8		3.6	2.3	2.3 (T0: 2.2)	2.3	2.8	2.9 (T0: 2)		(T0: 1.9)		4.0		2.8
LALRCQ (n=14)	3.2 (T0: 2)	3.2		3.3	3.0	3.2 (T0: 2.1)	3.3	3.0	3.2 (T0: 2.1)		(T0: 2.2)		4.4		4.3
LALWKF (n=14)	1.9 (T0: 1.2)	1.9		4.8	4.8	4.7 (T0: 3.2)	4.8	1.4	1.3 (T0: 1)		(T0: 1)		1.3		1.3
LAMABL (n=8)	1.8 (T0: 1)	1.9		3.6	5.8	5.2 (T0: 3.7)	5.6	1.9	1.8 (T0: 1)		(T0: 1)	(T0: 1.2)	1.8		3.5
LAMCKI (n=10)	2 (T0: 1)	3.9		3.6	2.7	2.3 (T0: 1)	2.0	3.8	4 (T0: 1.9)		(T0: 2.1)	(T0: 1.8)	3.9		3.9

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LAMCLE (n=14)	3 (T0: 2)	3.0		4.3	4.2	4.2 (T0: 2.9)	4.4	2.5	2.4 (T0: 2)		(T0: 2)	(T0: 2.1)	3.4		1.3
LAMCLF (n=9)	1.6 (T0: 1)	3.3		4.1	4.7	4.4 (T0: 2.8)	3.8	2.2	1.8 (T0: 2.1)		(T0: 1.9)	(T0: 1.4)	1.8		1.8
LAMEUB (n=15)	1.9 (T0: 1)	2.1		3.6	3.9	3.7 (T0: 2.2)	3.8	1.3	1.2 (T0: 1)		(T0: 1)		1.2		1.1
LAMEUU (n=13)	1.9 (T0: 0.9)	3.8		3.3	5.6	5.4 (T0: 2.8)	5.2	3.1	3.8 (T0: 1.9)		(T0: 1.9)		3.7		3.7
LAMFJO (n=15)	2 (T0: 1.1)	2.1		2.0	2.1	2 (T0: 0.9)	2.0	1.1	1.1 (T0: 0.5)		(T0: 0.8)		1.2		1.1
LAMIRY (n=10)	1.9 (T0: 1)	1.9		2.6	2.7	2.7 (T0: 2)	2.8	1.7	2 (T0: 2.3)		(T0: 2)		3.1		2.1
LAMJIC (n=9)	1.9 (T0: 1.1)	1.7		1.6	2.5	1.8 (T0: 1)	1.8	2.3	1.8 (T0: 1)		(T0: 1)		1.8		1.8
LAMPJB (n=13)	2.8 (T0: 2)	2.9		3.6	4.4	4.1 (T0: 3)	4.1	2.6	2.8 (T0: 2.1)		(T0: 1)	(T0: 1.8)	2.8		2.6
LAMQDL (n=14)	2 (T0: 1)	2.1		2.3	2.6	2.3 (T0: 1)	2.0	0.0	2.6 (T0: 2)		(T0: 1)	(T0: 1)	1.3		1.3
LAMQNE (n=14)	2.6 (T0: 1.1)	2.6		2.7	2.8	2.8 (T0: 1.3)	2.5	2.0	1.5 (T0: 1.1)		(T0: 0.9)	(T0: 1.9)	4.0		4.2
LAMQSF (n=14)	3.5 (T0: 2.1)	4.5		3.8	4.7	4.2 (T0: 3.2)	4.0	2.9	3.7 (T0: 3.9)		(T0: 4.2)	(T0: 2.2)	3.6		3.6
LAMRCO (n=14)	2.8 (T0: 1.9)	3.7		6.6	6.5	6 (T0: 4.1)	5.5	1.8	1.3 (T0: 1.1)		(T0: 1)	(T0: 2.1)	3.9		2.5
LAMRDS (n=15)	3.2 (T0: 1.9)	3.2		3.0	3.6	3 (T0: 1.8)	3.0	3.0	3 (T0: 2)		(T0: 1.9)	(T0: 1.7)	3.1		2.6
LAMRHL (n=14)	2 (T0: 1.3)	2.2		3.2	2.8	2.1 (T0: 1.5)	1.9	4.1	3.8 (T0: 2.7)		(T0: 1.3)	(T0: 1.3)	1.9		2.0
LAMRJK (n=15)	3 (T0: 2)	3.0		2.9	1.3	1.2 (T0: 0.9)	1.0	3.0	4.5 (T0: 2.9)		(T0: 1.9)	(T0: 1.7)	2.9		2.7
LAMRNQ (n=9)	2.2 (T0: 1.9)	2.1		2.2	2.3	2.3 (T0: 2.1)	2.1	2.5	2 (T0: 1.9)		(T0: 2)	(T0: 1.9)	2.0		2.7
LAMVUB (n=15)	2.1 (T0: 1.1)	2.1		2.1	2.7	2.1 (T0: 1)	2.1	0.6	0.8 (T0: 0.8)		(T0: 0.9)		1.1		0.8
LAMYDP (n=14)	3.9 (T0: 2)	4.0		3.7	4.2	3.8 (T0: 1.9)	3.9	3.2	3.4 (T0: 2.1)		(T0: 1.9)	(T0: 1.7)	3.3		3.2
LANBCH (n=14)	3.5 (T0: 1.9)	3.2		3.3	4.0	3.5 (T0: 2)	3.4	3.5	3 (T0: 1.8)		(T0: 2)		3.3		3.3
LANCEG (n=4)	4 (T0: 2.1)	4.0		3.5	5.9	5.4 (T0: 2.9)	3.6	3.6	4.1 (T0: 2)		(T0: 2)		4.1		1.9
LANCOX (n=10)	2.2 (T0: 1)	2.5		2.2	5.4	5.1 (T0: 4.3)	4.5	1.7	1.7 (T0: 1)		(T0: 1.1)		1.8		2.6
LANFEF (n=15)	2.1 (T0: 1.1)	2.0		2.3	2.6	2.1 (T0: 1)	2.1	1.0	0.7 (T0: 1.1)		(T0: 1)	(T0: 1.8)	2.9		2.9
LANMGC (n=15)	4.2 (T0: 1.9)	4.0		4.1	4.7	4.2 (T0: 2)	4.2	2.2	3.8 (T0: 1.9)		(T0: 2)		4.0		6.0
LANMOM (n=10)	1.4 (T0: 1.2)	1.4		3.0	2.7	2 (T0: 1.1)	1.5	3.5	2.9 (T0: 1.9)		(T0: 1.9)		2.9		4.8
LANPMZ (n=13)	2.1 (T0: 1.1)	2.1		2.0	2.3	2.2 (T0: 1)	2.2	2.0	2.1 (T0: 1.1)		(T0: 1.1)		2.1		1.9
LANTLE (n=15)	2.7 (T0: 2)	2.8		2.7	3.3	2.8 (T0: 2)	3.0	2.5	2.2 (T0: 2)		(T0: 1.9)		2.3		2.3
LANTSP (n=12)	1.4 (T0: 1)	1.3		2.9	3.7	3 (T0: 1.9)	3.0	0.6	1.5 (T0: 0.9)		(T0: 1.6)		2.5		3.3
LANUCB (n=14)	2 (T0: 1.1)	2.1		4.6	4.5	3.5 (T0: 2.1)	3.1	1.1	0.9 (T0: 2.1)		(T0: 2.8)		2.3		2.3
LAOBGQ (n=15)	1.4 (T0: 1)	1.4		6.1	5.2	4 (T0: 2.8)	4.2	1.6	2.7 (T0: 1.8)		(T0: 2.1)	(T0: 1.8)	2.8		4.0
LAOHLR (n=14)	1.9 (T0: 1.2)	4.9		4.0	4.3	3.7 (T0: 2)	3.1	2.1	1.7 (T0: 1.1)		(T0: 1)		1.7		1.7
LAOJAT (n=15)	2.2 (T0: 1.9)	3.4		3.2	3.8	3.4 (T0: 3)	3.3	2.3	2.2 (T0: 1.9)		(T0: 1.9)	(T0: 1.9)	2.3		1.2

LAOKLP (n=15)	2.1 (T0: 0.8)	2.1		2.1	2.5	2.2 (T0: 1)	2.1	1.4	1.2 (T0: 0.7)		(T0: 1)	(T0: 0.6)	1.2		1.2
LAOKTE (n=15)	3 (T0: 2)	2.8		3.5	3.5	3.3 (T0: 2.1)	3.2	3.0	2.4 (T0: 2)		(T0: 1.9)	(T0: 2.1)	2.5		2.4
LAPKLS (n=12)	3.4 (T0: 2.1)	3.1		3.6	4.2	3.1 (T0: 2.1)	3.2	2.5	1.9 (T0: 2)		(T0: 2.1)		2.0		2.1
LAPKXM (n=15)	3.2 (T0: 1.8)	3.2		3.8	4.2	3.3 (T0: 2)	3.4	3.7	2.9 (T0: 1.9)		(T0: 1.9)		3.6		0.0
LAPKZJ (n=15)	3.3 (T0: 1.4)	3.2		3.6	4.3	3.2 (T0: 1.9)	3.3	2.0	1.6 (T0: 2)		(T0: 1.9)		1.7		1.6
LAPWJD (n=14)	2.1 (T0: 1.1)	2.0		2.3	2.8	2.1 (T0: 1)	2.1	2.4	2 (T0: 1)		(T0: 1)		2.2		2.1
LAPWLP (n=14)	1.3 (T0: 1.1)	1.2		3.1	3.4	2.8 (T0: 1.9)	2.9	0.0	1.3 (T0: 1)		(T0: 1.1)		2.3		2.3
LAQYTA (n=15)	4.5 (T0: 2.8)	5.1		5.0	3.2	2.6 (T0: 2.1)	2.8	2.4	2.2 (T0: 1.9)		(T0: 2)	(T0: 2.4)	3.2		3.3
LAQYUT (n=15)	4.3 (T0: 1.9)	3.9		4.6	4.3	3 (T0: 2)	2.8	3.6	2.8 (T0: 1.6)		(T0: 1.9)	(T0: 0.6)	3.0		4.3
LAQYWQ (n=15)	2.6 (T0: 1.2)	2.8		5.6	5.9	4.5 (T0: 3)	4.6	3.2	2.7 (T0: 2)		(T0: 1.9)	(T0: 2.2)	2.6		1.4
LAQZME (n=12)	1.4 (T0: 1.2)	1.3		3.7	4.4	3.8 (T0: 2.3)	4.0	1.8	1.4 (T0: 1.1)		(T0: 1.2)	(T0: 1.2)	1.4		1.3

Table 41: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, dc: all T1 plants where the average of all copy number assays listed in this table was 3.51-4.49, tc: all T1 plants where the average of all copy number assays listed in this table was 5.51-6.49. The number of T1 plants fullfilling these criteria are displayed in parentheses.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay A1 located near the left T-DNA border and assay A13 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.							Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay A1 located near the left T-DNA border and assay A13 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p LuCnl 2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)

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sc (n=296)	1.9		2.1		2.6	2.7	2.4		2.3	1.5	1.6						1.8		1.7								
dc (n=198)	3.4		3.7		4.2	4.8	4.2		4.2	3.2	3.4						3.8		3.6								
tc (n=2)	4.0		5.6		7.6	7.5	7.1		6.3	4.6	4.4						5.9		4.7								

Table 42: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LALHCY (n=15)	4.9 ± 0.1	0.3 ± 0	0 ± 0	4 ± 0.2	23.7 ± 1	32 ± 0.9	1.2 ± 0.1	4.2 ± 0.4	2.2 ± 0.2	0.3 ± 0	0.8 ± 0	0.3 ± 0.3	0.2 ± 0	0.1 ± 0	4.4 ± 1.4	2.5 ± 0.7	2.9 ± 0.4	8.1 ± 0.8	0.3 ± 0	0 ± 0	1.3 ± 0.1	3.1 ± 0.3	0 ± 0	1.9 ± 0.2	1.2 ± 0.5	
LALIAO (n=15)	5 ± 0.2	0.2 ± 0	0 ± 0	2.9 ± 0.4	26.8 ± 3.4	30.8 ± 1.6	0.6 ± 0.2	5.5 ± 0.9	0.9 ± 0.4	0.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.2 ± 0.7	0.4 ± 0.3	2.5 ± 0.4	1.6 ± 0.1	3.7 ± 1.3	9.9 ± 2.5	0.3 ± 0.1	0 ± 0	0.7 ± 0.3	3.3 ± 1	0 ± 0	1.2 ± 0.4	0.2 ± 0.3	0.6 ± 0.2
LALKA (n=15)	5.4 ± 0.2	0.3 ± 0	0 ± 0	2.7 ± 0.5	25.3 ± 2.2	36.4 ± 1.1	0.6 ± 0.1	5.7 ± 0.5	2.2 ± 0.3	0.5 ± 0.1	0.7 ± 0.1	0.6 ± 0	0.1 ± 0.1	0.1 ± 0	1.7 ± 1	1 ± 0.5	2.2 ± 0.5	6.9 ± 1.5	0.3 ± 0	0 ± 0	1.3 ± 0.2	3.4 ± 0.9	0 ± 0	1.8 ± 0.4	0.6 ± 0.5	
LALLTL (n=11)	5 ± 0.2	0.3 ± 0	0 ± 0	3.6 ± 0.3	28.5 ± 4.4	31.5 ± 1.1	0.8 ± 0.2	4.6 ± 0.5	1.3 ± 0.4	0.2 ± 0	0.8 ± 0	0.6 ± 0.3	0.6 ± 0.2	0.2 ± 0.1	2.6 ± 1	1.3 ± 0.6	6 ± 2.4	6.2 ± 1.5	0.3 ± 0	0 ± 0	1.1 ± 0.4	2.4 ± 0.6	0 ± 0.1	1.3 ± 0.4	0.6 ± 0.5	
LALQAM (n=15)	5.4 ± 0.8	0.3 ± 0.1	0 ± 0	3.8 ± 0.2	23.3 ± 1.4	32.7 ± 1	0.5 ± 0.2	3.8 ± 0.3	1 ± 0.4	0.1 ± 0	0.8 ± 0	0.7 ± 0.1	1.5 ± 0.9	0.4 ± 0.2	3.5 ± 0.8	1.5 ± 0.2	6.4 ± 1.4	8 ± 1.3	0.3 ± 0	0 ± 0	0.9 ± 0.3	2.9 ± 0.7	0 ± 0	1.6 ± 0.4	0.3 ± 0.1	
LALQDS (n=14)	5 ± 0.1	0.3 ± 0	0 ± 0	3.2 ± 0.4	29.1 ± 2.4	35.7 ± 1.1	0.6 ± 0.1	6.2 ± 0.6	1 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	1.5 ± 0.5	1 ± 0.2	2.4 ± 0.6	6.7 ± 0.7	0.3 ± 0	0 ± 0	0.7 ± 0.1	2.7 ± 0.3	0 ± 0	1 ± 0.2	0.3 ± 0.1	
LALRCQ (n=14)	5.1 ± 0.1	0.3 ± 0	0 ± 0	4 ± 0.5	23 ± 1.1	33.2 ± 1.2	1 ± 0.2	3.5 ± 0.5	2.3 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.6 ± 0	0.2 ± 0.1	0 ± 0	5.1 ± 1.6	1.7 ± 0.4	4.1 ± 0.7	6.6 ± 0.9	0.3 ± 0	0 ± 0	1.9 ± 0.3	2.8 ± 0.4	0 ± 0	1.8 ± 0.3	1.1 ± 0.4	
LALWKF (n=14)	5.3 ± 0.1	0.3 ± 0.1	0 ± 0	3.7 ± 0.3	26.7 ± 6.7	27.6 ± 3.6	1.3 ± 0.4	3.3 ± 0.3	2.2 ± 0.7	0.3 ± 0.1	0.8 ± 0	0.7 ± 0.1	0.6 ± 0.5	0.2 ± 0.2	2.1 ± 0.5	0.9 ± 0.3	9.3 ± 2.7	8.8 ± 1.5	0.3 ± 0.1	0 ± 0	0.7 ± 0.3	2.9 ± 0.6	0 ± 0	1.6 ± 0.5	0.1 ± 0.1	
LAMABL (n=8)	4.8 ± 0.2	0.3 ± 0	0 ± 0	3.3 ± 0.5	27.8 ± 6.3	30 ± 3.8	1.4 ± 0.2	3.7 ± 0.5	2.7 ± 0.7	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.1	0 ± 0	1.3 ± 0.4	0.6 ± 0.1	7 ± 0.8	8.6 ± 1.7	0.3 ± 0	0 ± 0	1.3 ± 0.3	3.2 ± 0.4	0 ± 0	1.4 ± 0.3	0.1 ± 0.1	
LAMCKI (n=10)	5.1 ± 0.1	0.2 ± 0	0 ± 0	3.5 ± 0.2	26.4 ± 0.7	33.8 ± 0.5	1.1 ± 0	4.2 ± 0.2	1.8 ± 0.2	0.2 ± 0	0.8 ± 0	0.6 ± 0	0.1 ± 0	0 ± 0	3.9 ± 0.6	1.7 ± 0.2	2.9 ± 0.2	6.8 ± 0.6	0.3 ± 0	0 ± 0	1.2 ± 0.1	2.7 ± 0.3	0 ± 0	1.3 ± 0.1	0.9 ± 0.1	
LAMCLE (n=14)	4.9 ± 0.2	0.2 ± 0.1	0 ± 0	3.4 ± 0.2	25.1 ± 2.4	32.2 ± 1.6	1.1 ± 0.3	3.5 ± 0.3	2.4 ± 0.6	0.3 ± 0.1	0.8 ± 0	0.7 ± 0.1	0.4 ± 0.2	0 ± 0.1	2.3 ± 0.5	1 ± 0.2	5.8 ± 1.3	8.4 ± 1.3	0.1 ± 0.1	0 ± 0	1.8 ± 0.4	3.4 ± 0.6	0 ± 0	1.8 ± 0.5	0.1 ± 0.2	

LAMCLF (n=9)	5.1 ± 0.1	0.3 ± 0	0 ± 0	3.6 ± 0.1	24.7 ± 3.5	28.5 ± 2	1.1 ± 0.6	2.8 ± 0.5	2 ± 0.6	0.2 ± 0.1	0.8 ± 0	0.6 ± 0.1	1.4 ± 0.7	0.1 ± 0.1	2.8 ± 0.7	1 ± 0.2	9.7 ± 2.2	9.4 ± 1	0.3 ± 0	0 ± 0	1 ± 0.3	2.8 ± 0.5	0 ± 0	1.5 ± 0.3	0 ± 0.1	
LAMEUB (n=15)	5 ± 0.2	0.3 ± 0.1	0 ± 0	3.8 ± 0.5	28.4 ± 5.6	29.7 ± 2.1	0.9 ± 0.3	3.4 ± 0.6	1.3 ± 0.4	0.1 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	1 ± 0.4	0.2 ± 0.1	2.7 ± 0.3	1.1 ± 0.3	8.4 ± 2.9	7.6 ± 1.9	0.3 ± 0	0 ± 0	0.5 ± 0.2	2.2 ± 0.7	0 ± 0	1.2 ± 0.5	0.1 ± 0.1	
LAMEUU (n=13)	5 ± 0.1	0.1 ± 0.1	0 ± 0	3.3 ± 0.4	25.8 ± 4.8	34.3 ± 1.8	1.4 ± 0.3	4.2 ± 0.7	2.1 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.2	1.8 ± 1.8	1.4 ± 1.5	1.2 ± 0.3	3.1 ± 0.8	7.8 ± 1.1	0.2 ± 0.2	0 ± 0	1.4 ± 0.2	3 ± 0.4	0 ± 0	1.5 ± 0.3	0.3 ± 0.3	
LAMFJO (n=15)	5.1 ± 0.7	0.2 ± 0.1	0 ± 0	3.5 ± 0.7	26.5 ± 1.9	32.9 ± 0.8	0.2 ± 0.1	4.5 ± 0.4	0.4 ± 0.1	0 ± 0	0.9 ± 0.1	1 ± 0.1	2.2 ± 0.7	1.7 ± 1.9	3.7 ± 0.6	2 ± 0.3	4.7 ± 1.8	6.2 ± 0.7	0.3 ± 0.1	0 ± 0	0.3 ± 0.2	2.2 ± 0.3	0 ± 0.1	1 ± 0.2	0.4 ± 0.3	
LAMIRY (n=10)	5 ± 0.2	0.3 ± 0	0 ± 0	3.5 ± 0.3	26.9 ± 6.8	30.9 ± 2.8	0.6 ± 0.1	4.6 ± 0.5	1.2 ± 0.3	0.2 ± 0	0.8 ± 0	0.7 ± 0.2	1.1 ± 0.2	0.4 ± 0.1	2.4 ± 0.4	1.4 ± 0.3	4.5 ± 0.9	9.1 ± 2.1	0.3 ± 0	0 ± 0	1 ± 0.2	3 ± 0.6	0 ± 0	1.6 ± 0.4	0.5 ± 0.1	
LAMJIC (n=9)	5.8 ± 0.4	0.2 ± 0.1	0 ± 0	3.1 ± 0.5	28.5 ± 6.6	33.1 ± 3.9	0.6 ± 0.2	5.3 ± 1.2	1.3 ± 0.6	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	2.6 ± 0.4	1.3 ± 0.3	3.9 ± 1.3	6.2 ± 1.8	0.3 ± 0.1	0 ± 0	0.9 ± 0.3	2.6 ± 1	0 ± 0	1.4 ± 0.4	0.4 ± 0.1	
LAMPJB (n=13)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3.1 ± 0.3	24.1 ± 1.7	33.6 ± 0.9	0.9 ± 0.2	3.7 ± 0.5	2.2 ± 0.5	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.4 ± 0.3	0 ± 0	2.8 ± 0.4	1.2 ± 0.2	5.5 ± 2.2	8.7 ± 1	0 ± 0.1	0 ± 0	1.5 ± 0.3	3.2 ± 0.4	0 ± 0	2 ± 0.3	0.1 ± 0.3	
LAMQDL (n=14)	5 ± 0.5	0.2 ± 0	0 ± 0	2.7 ± 0.4	26.4 ± 2.2	31.2 ± 1	0.2 ± 0	5.2 ± 0.6	0.4 ± 0.1	0.1 ± 0	0.7 ± 0.1	1 ± 0.1	2.8 ± 0.5	1 ± 0.2	3.7 ± 0.4	2.1 ± 0.4	4.7 ± 2	6.7 ± 1.2	0.3 ± 0	0 ± 0	0.4 ± 0.1	2.7 ± 0.7	0 ± 0	0.8 ± 0.2	0.4 ± 0.2	1.1 ± 0.1
LAMQNE (n=14)	6.9 ± 1.3	0.3 ± 0.1	0 ± 0	2.7 ± 0.3	27.8 ± 2.6	31.8 ± 0.7	0.8 ± 0.1	4.9 ± 0.6	1.3 ± 0.1	0.2 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0.2 ± 0.1	4 ± 1.7	1.9 ± 0.8	2.7 ± 1	5.9 ± 1.1	0.3 ± 0	0 ± 0	1.2 ± 0.2	2.8 ± 0.6	0 ± 0	1.2 ± 0.3	0.8 ± 0.6	0.4 ± 0.1
LAMQSF (n=14)	5.2 ± 0.1	0.3 ± 0	0 ± 0	3.4 ± 0.3	24.3 ± 2.7	31.5 ± 1.2	0.8 ± 0.4	4.5 ± 0.7	2 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.6 ± 0.2	0.2 ± 0.2	1.9 ± 0.6	1.3 ± 0.5	4.1 ± 1.5	10.6 ± 1.7	0.3 ± 0	0 ± 0	1.1 ± 0.2	3.6 ± 0.5	0 ± 0	1.8 ± 0.5	0.3 ± 0.3	
LAMRCO (n=14)	5.5 ± 0.6	0.3 ± 0.1	0 ± 0	4.4 ± 0.6	23.9 ± 6.8	28.4 ± 3.1	1.3 ± 0.4	2.7 ± 0.4	3.4 ± 0.8	0.3 ± 0.1	1 ± 0.2	0.6 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	3.7 ± 0.8	1.3 ± 0.3	5.7 ± 1.3	8.7 ± 1.7	0.4 ± 0.1	0 ± 0	1.8 ± 0.4	3.3 ± 0.8	0 ± 0	2.1 ± 0.6	0.4 ± 0.2	0.4 ± 0.1
LAMRDS (n=15)	4.8 ± 0.1	0 ± 0	0 ± 0	3.6 ± 0.4	23.4 ± 1.7	32.7 ± 1.3	1.1 ± 0.2	3.4 ± 0.5	2.3 ± 0.3	0.2 ± 0	0.9 ± 0.1	0.7 ± 0	0.2 ± 0.1	5.4 ± 0.9	0 ± 0.1	1.2 ± 0.1	3.4 ± 0.8	8.2 ± 0.7	0.4 ± 0.1	0 ± 0	2 ± 0.4	3.5 ± 0.3	0 ± 0	1.8 ± 0.3	0.5 ± 0.3	
LAMRHL (n=14)	6 ± 1	0.3 ± 0.1	0 ± 0	3.5 ± 0.5	26.5 ± 6	26.2 ± 3.9	0.6 ± 0.1	3.7 ± 0.8	1 ± 0.3	0.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.2	1.6 ± 0.6	0.4 ± 0.1	3 ± 0.6	1.9 ± 0.4	4.6 ± 0.8	10.5 ± 1.9	0.4 ± 0	0 ± 0	1 ± 0.3	3.9 ± 1	0 ± 0	1.9 ± 0.5	0.3 ± 0.2	1.4 ± 0.4
LAMRJK (n=15)	4.9 ± 0.2	0.1 ± 0	0 ± 0	3.6 ± 0.4	26.4 ± 2.9	33 ± 1	1 ± 0.2	4.1 ± 0.6	2 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0 ± 0.1	0.5 ± 1.4	2.6 ± 1.4	1.3 ± 0.3	4.4 ± 1.3	7.7 ± 1.2	0.1 ± 0.2	0 ± 0	1.3 ± 0.4	3.2 ± 0.6	0 ± 0	1.5 ± 0.2	0.2 ± 0.4	
LAMRNQ (n=9)	4.8 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.4	29.6 ± 3.8	32.2 ± 0.9	0.5 ± 0.1	5.1 ± 0.8	0.9 ± 0.2	0.1 ± 0	0.8 ± 0.1	0.9 ± 0	1.2 ± 0.2	0.3 ± 0.1	2.8 ± 0.7	1.3 ± 0.3	4.6 ± 1.3	6 ± 1.2	0.3 ± 0	0 ± 0	0.9 ± 0.1	2.4 ± 0.3	0 ± 0	1.2 ± 0.3	0.4 ± 0.2	0.6 ± 0.2
LAMVUB (n=15)	4.9 ± 0.3	0.2 ± 0	0 ± 0	3.6 ± 0.4	24.6 ± 1.9	33.4 ± 0.5	0.2 ± 0.1	4.8 ± 0.4	0.4 ± 0.1	0 ± 0	0.9 ± 0.1	1 ± 0.1	2.8 ± 0.5	1 ± 0.2	4 ± 0.4	2.4 ± 0.3	4 ± 1.4	6.7 ± 1	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.4	0 ± 0	1.3 ± 0.3	0.6 ± 0.2	
LAMYDP (n=14)	5.1 ± 0.5	0.2 ± 0.1	0 ± 0	4 ± 0.8	23.1 ± 2.3	31.7 ± 1.1	1.2 ± 0.1	4.1 ± 0.5	2.4 ± 0.3	0.3 ± 0	0.9 ± 0.1	0.6 ± 0	0.3 ± 0.1	0 ± 0	2.5 ± 0.4	1.3 ± 0.3	4.1 ± 0.8	10.3 ± 1.6	0.3 ± 0.1	0 ± 0	1.3 ± 0.3	3.6 ± 0.7	0 ± 0	2.1 ± 0.5	0.4 ± 0.2	
LANBCH (n=14)	4.7 ± 0.8	0.2 ± 0.1	0 ± 0	3.4 ± 0.4	25 ± 3.9	32.7 ± 1.5	0.8 ± 0.2	4.7 ± 0.5	1.5 ± 0.5	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.2	0.2 ± 0.1	2.3 ± 0.4	1.5 ± 0.2	3.5 ± 0.5	9.2 ± 1.8	0.3 ± 0	0 ± 0	1.3 ± 0.4	3.7 ± 0.9	0 ± 0	2 ± 0.6	0.5 ± 0.1	0.3 ± 0

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LANCEG (n=4)	4.9 ± 0.1	0.1 ± 0.1	0 ± 0	3.3 ± 0.2	23.8 ± 1.2	33.2 ± 0.5	0.9 ± 0.1	3.7 ± 0.4	2 ± 0.3	0.3 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0.1	5.5 ± 0.2	0.2 ± 0.1	0.7 ± 0.1	1.5 ± 0.4	10.8 ± 0.4	0.3 ± 0.1	0 ± 0	1.2 ± 0.1	3.8 ± 0.2	0 ± 0	1.7 ± 0.2	0 ± 0	
LANCOX (n=10)	5.1 ± 0.7	0.2 ± 0.1	0 ± 0	3.1 ± 0.2	25 ± 2.5	35.5 ± 1.3	0.7 ± 0.1	4.1 ± 0.3	2.1 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0 ± 0	7.2 ± 2.4	0.2 ± 0.1	0.6 ± 0.1	1.6 ± 0.3	7.3 ± 1.5	0.3 ± 0.1	0 ± 0	0.9 ± 0.4	2.7 ± 0.7	0 ± 0.1	1.4 ± 0.5	0 ± 0	
LANFEF (n=15)	5.1 ± 0.3	0.1 ± 0.1	0 ± 0	3.5 ± 0.4	27.2 ± 1	34.3 ± 1.1	0.2 ± 0.1	4.5 ± 0.9	0.3 ± 0	0 ± 0	0.8 ± 0.1	1 ± 0	2.5 ± 0.3	0.8 ± 0.2	3.5 ± 0.5	1.6 ± 0.4	3.3 ± 1.4	5.5 ± 0.5	0.2 ± 0.1	0 ± 0	1 ± 0.1	2.2 ± 0.3	0 ± 0	1.1 ± 0.1	0.8 ± 0.2	0.9 ± 0
LANMGC (n=15)	5.6 ± 0.9	0.2 ± 0.1	0 ± 0	4 ± 0.5	25.1 ± 1.9	33.1 ± 1.8	1 ± 0.2	4.3 ± 0.3	2.1 ± 0.2	0.2 ± 0	0.9 ± 0.1	0.7 ± 0	0.2 ± 0	0 ± 0	7.9 ± 1.3	3.6 ± 0.7	1.4 ± 0.5	3.7 ± 1.3	0.3 ± 0	0 ± 0	0.7 ± 0.2	1.5 ± 0.6	0 ± 0	0.8 ± 0.4	2.4 ± 0.6	
LANMOM (n=10)	5.4 ± 0.4	0.2 ± 0	0 ± 0	2.8 ± 0.2	26.9 ± 3.1	31.8 ± 1.3	0.8 ± 0.2	4.9 ± 0.5	1.4 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.6 ± 0.3	0.2 ± 0.1	2 ± 0.3	1.2 ± 0.2	3.9 ± 0.5	8.6 ± 1.5	0.3 ± 0	0 ± 0	1.2 ± 0.2	3.7 ± 0.7	0 ± 0	1.5 ± 0.4	0.4 ± 0.1	0.3 ± 0.1
LANPMZ (n=13)	4.8 ± 0.6	0.2 ± 0.1	0 ± 0	3.7 ± 0.3	26.2 ± 1.4	34.4 ± 1	0.3 ± 0.1	5 ± 0.4	0.5 ± 0.1	0.1 ± 0	0.9 ± 0	0.9 ± 0.1	1.5 ± 0.3	0.5 ± 0.2	3 ± 0.2	2 ± 0.3	2.7 ± 0.3	6.9 ± 0.8	0.4 ± 0	0 ± 0	0.8 ± 0.2	2.8 ± 0.6	0 ± 0	1.5 ± 0.3	0.7 ± 0.1	1 ± 0.1
LANTLE (n=15)	5 ± 0.4	0.2 ± 0.1	0 ± 0	2.4 ± 0.4	26.7 ± 4	32.7 ± 1.2	0.4 ± 0.3	6.1 ± 0.8	1 ± 0.5	0.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.2	1.4 ± 1	0.5 ± 0.5	2.5 ± 0.5	1.7 ± 0.4	3.9 ± 1.4	7.9 ± 1.9	0 ± 0.1	0 ± 0	0.7 ± 0.4	3.3 ± 0.8	0 ± 0	1.5 ± 0.4	0.1 ± 0.2	0.4 ± 0.2
LANTSP (n=12)	4.9 ± 0.2	0.2 ± 0	0.1 ± 0	3.3 ± 0.3	35.9 ± 6.9	26.8 ± 4	0.8 ± 0.2	4.6 ± 0.9	1.1 ± 0.4	0.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.3	0.2 ± 0.1	2.1 ± 0.4	1.2 ± 0.2	3.4 ± 0.9	6.6 ± 1.8	0.4 ± 0	0 ± 0	1 ± 0.4	2.5 ± 0.7	0 ± 0	1.3 ± 0.4	0.4 ± 0.2	0.4 ± 0.3
LANUCB (n=14)	5 ± 0.5	0.3 ± 0	0 ± 0	2.9 ± 0.4	28.2 ± 4.5	28.8 ± 2.2	1.5 ± 0.6	3.8 ± 0.8	2.7 ± 1.5	0.4 ± 0.3	0.7 ± 0.1	0.7 ± 0.1	0.5 ± 0.5	0.1 ± 0.1	2.2 ± 0.7	1.1 ± 0.3	5.7 ± 1.9	9.2 ± 1.4	0.2 ± 0.1	0 ± 0	0.9 ± 0.3	3.3 ± 0.5	0 ± 0	1.3 ± 0.4	0 ± 0.1	0.6 ± 0.5
LAOBGQ (n=15)	5.2 ± 0.4	0.2 ± 0.1	0 ± 0	3.5 ± 0.3	31.8 ± 7.9	29.2 ± 4.2	1.2 ± 0.4	3.9 ± 0.6	2.5 ± 0.7	0.4 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.2 ± 0.2	0 ± 0	2.1 ± 0.9	1.1 ± 0.5	3.9 ± 0.6	7.3 ± 1.5	0.2 ± 0.2	0 ± 0	1.1 ± 0.3	2.9 ± 0.6	0 ± 0	1.4 ± 0.4	0.1 ± 0.3	0.1 ± 0.1
LAOHLR (n=14)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3.5 ± 0.3	26.5 ± 6.1	33 ± 2.7	1.1 ± 0.2	3.7 ± 0.7	2.1 ± 0.4	0.2 ± 0	0.9 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	4.1 ± 1.6	1.4 ± 0.5	5.3 ± 0.8	6.8 ± 1.2	0.3 ± 0.1	0 ± 0	0.8 ± 0.2	2.3 ± 0.4	0 ± 0	1.2 ± 0.3	0.3 ± 0.3	
LAOJAT (n=15)	5.1 ± 0.5	0.1 ± 0.1	0 ± 0	2.8 ± 0.7	28.7 ± 4	34 ± 1.8	0.6 ± 0.1	5 ± 0.8	1.3 ± 0.4	0.1 ± 0.1	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.4	0.1 ± 0.2	1.5 ± 0.5	0.9 ± 0.2	4.3 ± 0.7	7.9 ± 1.3	0 ± 0	0 ± 0	0.8 ± 0.3	2.9 ± 0.4	0 ± 0	1.4 ± 0.3	0 ± 0	0.3 ± 0.1
LAOKLP (n=15)	4.7 ± 0.4	0.2 ± 0	0 ± 0	3.1 ± 0.2	26 ± 2.2	32.5 ± 1.3	0.2 ± 0	5.2 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0	1 ± 0	2.5 ± 0.7	1 ± 0.2	3.5 ± 0.4	2.1 ± 0.3	4.4 ± 1.6	6.5 ± 0.9	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.5 ± 0.6	0 ± 0	1.2 ± 0.3	0.5 ± 0.2	1.1 ± 0.6
LAOKTE (n=15)	4.7 ± 0.3	0.2 ± 0.1	0.1 ± 0	2.8 ± 0.4	27.1 ± 7.8	31.4 ± 2.3	0.5 ± 0.3	5.5 ± 1.7	0.9 ± 0.6	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.2 ± 0.7	0.4 ± 0.2	2.5 ± 0.8	1.6 ± 0.4	4.1 ± 2.2	8.2 ± 2.9	0.3 ± 0.1	0 ± 0	0.9 ± 0.4	3.3 ± 1	0 ± 0	1.6 ± 0.6	0.4 ± 0.2	0.5 ± 0.2
LAPKLS (n=12)	5.2 ± 0.3	0.2 ± 0.1	0 ± 0	3.7 ± 0.5	23.8 ± 1.9	33.6 ± 0.7	0.9 ± 0.1	4.1 ± 0.5	1.9 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.4 ± 0.3	0.1 ± 0.1	3.1 ± 0.5	1.5 ± 0.4	6 ± 2.3	7.9 ± 1.5	0.2 ± 0.1	0 ± 0	0.8 ± 0.3	2.6 ± 0.7	0 ± 0	1.4 ± 0.5	0.3 ± 0.3	0.4 ± 0.2
LAPKXM (n=15)	4.6 ± 0.3	0.2 ± 0	0 ± 0	3.2 ± 0.3	26.1 ± 2	32 ± 0.9	0.7 ± 0.2	5.2 ± 0.5	1.2 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.7	0.3 ± 0.3	2.3 ± 0.4	1.7 ± 0.2	3.1 ± 0.7	9.8 ± 2	0.3 ± 0	0 ± 0	0.9 ± 0.3	3.4 ± 0.7	0 ± 0	1.4 ± 0.4	0.4 ± 0.3	0.5 ± 0.2
LAPKZJ (n=15)	5.1 ± 0.5	0.1 ± 0.1	0 ± 0	3.6 ± 0.4	24.6 ± 1.9	31.8 ± 1.6	0.4 ± 0.2	4.7 ± 0.7	0.6 ± 0.3	0 ± 0	0.8 ± 0.1	0.9 ± 0.2	2.1 ± 0.8	0.8 ± 0.4	3.4 ± 0.5	2.1 ± 0.5	4.9 ± 2.1	7.7 ± 1.1	0.2 ± 0.2	0 ± 0	0.6 ± 0.3	2.8 ± 0.5	0 ± 0	1.4 ± 0.3	0.4 ± 0.5	1 ± 0.2
LAPWJD (n=14)	5.1 ± 0.4	0.1 ± 0.1	0 ± 0	3.6 ± 0.1	27 ± 0.9	34 ± 0.7	0.3 ± 0.1	4.9 ± 0.2	0.5 ± 0.1	0 ± 0	0.8 ± 0.1	0.9 ± 0.1	1.6 ± 0.4	0.6 ± 0.2	3.4 ± 0.2	2.2 ± 0.2	2.5 ± 0.3	6.2 ± 0.3	0.2 ± 0.2	0 ± 0	0.7 ± 0.1	2.5 ± 0.2	0 ± 0	1.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.1

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LAPWLP (n=14)	4.5 ± 0.4	0.2 ± 0	0 ± 0	3 ± 0.3	32.5 ± 6.3	23.1 ± 3.7	0.5 ± 0.3	5.1 ± 0.8	0.5 ± 0.3	0.1 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	2.5 ± 0.9	0.9 ± 0.4	2.2 ± 0.4	1.8 ± 0.4	3.5 ± 0.7	10 ± 2.3	0.3 ± 0	0 ± 0	0.7 ± 0.2	3.6 ± 1	0 ± 0	1.7 ± 0.6	0.2 ± 0.2	1.3 ± 0.4
LAQYTA (n=15)	5.6 ± 0.2	0 ± 0	0 ± 0	3.3 ± 0.3	24.7 ± 1.3	31.5 ± 1.3	1.3 ± 0.2	4.1 ± 0.5	2.1 ± 0.3	0.1 ± 0.1	0.7 ± 0.1	0.6 ± 0	0.2 ± 0.2	0 ± 0	3.6 ± 1.6	1.6 ± 0.8	5.8 ± 2.6	8.2 ± 1.4	0 ± 0	0 ± 0	1 ± 0.2	2.9 ± 0.5	0 ± 0	1.4 ± 0.3	0.5 ± 0.7	0.5 ± 0.2
LAQYUT (n=15)	5.5 ± 0.3	0.2 ± 0	0 ± 0	3.3 ± 0.3	26.6 ± 4	33.4 ± 1.5	1 ± 0.2	4 ± 0.6	2.2 ± 0.5	0.2 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0 ± 0	3.7 ± 2.1	1.5 ± 0.8	4.1 ± 1.4	7.1 ± 1.5	0.1 ± 0.1	0 ± 0	1.1 ± 0.2	2.4 ± 0.5	0 ± 0	1.3 ± 0.3	0.5 ± 0.6	0.2 ± 0.2
LAQYWQ (n=15)	5.6 ± 0.2	0.2 ± 0.1	0 ± 0	3.9 ± 0.5	26.9 ± 6	30.3 ± 2.1	1.3 ± 0.4	4.1 ± 0.6	2.5 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.2 ± 0.2	0 ± 0	2.3 ± 1.1	1.1 ± 0.6	6.5 ± 3	7.9 ± 2	0.1 ± 0.1	0 ± 0	0.8 ± 0.4	2.5 ± 0.7	0 ± 0	1.4 ± 0.6	0.2 ± 0.4	0.2 ± 0.2
LAQZME (n=12)	5.3 ± 0.7	0.2 ± 0.1	0 ± 0	2.5 ± 0.2	33.3 ± 6.9	24.1 ± 4.6	0.5 ± 0.1	4.1 ± 0.5	0.9 ± 1	0.2 ± 0.1	0.7 ± 0.1	1.1 ± 0.4	1.3 ± 1.1	0.6 ± 0.3	2.7 ± 0.2	1.8 ± 0.3	5.6 ± 0.5	8.2 ± 1.2	0.3 ± 0	0 ± 0	0.7 ± 0.3	3 ± 0.5	0 ± 0	1.6 ± 0.6	0.1 ± 0.1	1.4 ± 1.1

Table 43: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LALHCY (n=1)	7.6	1.1	0.7	2.3	19.7	25.6	0.9	6.3	3.6	0.7	0.8	0.6	0.0	0.0	1.2	1.1	3.9	10.4	0.0	0.0	1.0	7.0	0.0	3.4	0.0	0.0
LALIAO (n=1)	5.1	0.2	0.0	3.1	23.3	31.4	0.8	4.2	1.4	0.2	0.8	0.7	0.7	0.1	2.4	1.5	5.7	13.2	0.4	0.0	0.6	2.7	0.0	1.1	0.0	0.4
LALJKA (n=1)	5.6	0.3	0.0	2.1	22.4	34.6	0.5	6.2	2.6	0.6	0.6	0.6	0.2	0.1	1.0	0.7	2.9	9.4	0.3	0.0	1.6	4.9	0.0	2.3	0.4	
LALLTL (n=1)	5.4	0.3	0.0	3.6	24.5	31.8	0.9	5.0	1.7	0.3	0.8	0.0	0.3	0.2	2.8	1.9	2.8	8.6	0.3	0.0	1.7	3.7	0.0	2.2	1.1	
LALQAM (n=1)	7.1	0.5	0.0	3.9	21.1	30.2	0.8	4.0	1.7	0.2	0.8	0.5	0.5	0.2	2.3	1.2	6.2	10.4	0.3	0.0	1.3	4.3	0.0	2.2	0.3	0.0
LALQDS (n=1)	5.3	0.3	0.0	3.4	25.3	35.7	0.7	6.1	1.4	0.3	0.8	0.0	0.3	0.2	1.6	1.2	2.4	8.3	0.4	0.0	0.9	3.4	0.0	1.5	0.4	0.0
LALRCQ (n=1)	5.0	0.3	0.0	3.6	23.0	32.7	1.0	4.0	2.3	0.3	0.8	0.6	0.3	0.1	3.5	1.5	3.8	8.4	0.3	0.0	2.0	3.5	0.0	2.1	0.9	0.0
LALWKF (n=1)	5.5	0.3	0.0	3.7	23.0	29.0	1.4	3.4	2.9	0.4	0.8	0.6	0.3	0.1	1.9	1.2	5.9	11.3	0.3	0.0	1.2	3.9	0.0	2.4	0.2	0.0
LAMABL (n=1)	4.6	0.3	0.0	3.7	24.3	31.5	1.4	3.4	2.8	0.3	0.9	0.6	0.2	0.0	1.1	0.6	7.3	10.1	0.3	0.0	1.2	3.6	0.0	1.5	0.0	0.0
LAMCKI (n=1)	5.1	0.2	0.0	3.4	25.6	33.0	1.1	4.2	2.0	0.3	0.8	0.6	0.1	0.0	3.9	1.8	3.1	7.7	0.3	0.0	1.3	3.0	0.0	1.5	0.9	
LAMCLE (n=1)	4.8	0.2	0.0	3.9	22.2	31.9	1.3	3.3	3.1	0.4	0.9	0.6	0.0	0.0	2.0	1.1	3.8	10.5	0.0	0.0	2.8	4.3	0.0	2.8	0.0	0.0
LAMCLF (n=1)	5.2	0.3	0.0	3.8	24.0	27.7	1.7	3.1	3.1	0.3	0.8	0.5	0.7	0.0	1.9	0.9	7.5	11.2	0.3	0.0	1.3	3.6	0.0	1.9	0.2	0.0
LAMEUB (n=1)	5.4	0.4	0.0	4.7	26.2	27.5	1.0	3.4	1.5	0.2	1.0	0.7	1.2	0.3	2.6	1.8	3.8	11.1	0.3	0.0	0.8	3.6	0.0	2.2	0.3	0.0
LAMEUU (n=1)	10.9	1.2	1.0	2.9	16.4	27.3	0.0	8.4	2.3	0.7	1.0	0.7	0.0	0.0	1.1	0.7	4.5	6.6	0.3	0.0	1.4	6.3	0.0	4.5	0.0	0.0
LAMFJO (n=1)	5.5	0.4	0.0	2.4	26.8	30.6	0.2	7.3	0.4	0.1	0.6	0.8	2.1	1.0	1.7	1.4	2.8	7.4	0.2	0.0	0.9	4.5	0.0	1.8	0.5	0.5
LAMIRY (n=1)	5.1	0.3	0.0	3.7	23.0	30.3	0.7	4.5	1.4	0.2	0.8	0.7	1.1	0.3	2.4	1.6	4.4	11.6	0.3	0.0	1.1	3.7	0.0	2.0	0.5	0.0

LAMJIC (n=1)	6.3	0.3	0.0	2.9	24.5	28.3	0.7	6.4	1.3	0.3	0.7	0.7	0.7	0.2	2.7	1.7	4.9	9.4	0.3	0.0	1.1	4.3	0.0	1.8	0.5	0.0
LAMPJB (n=1)	4.9	0.2	0.0	3.2	24.2	32.0	1.1	4.0	2.7	0.4	0.8	0.7	0.0	0.0	2.2	1.1	4.0	10.3	0.0	0.0	1.8	4.0	0.0	2.4	0.0	0.0
LAMQDL (n=1)	4.3	0.2	0.0	2.4	24.7	28.5	0.2	6.1	0.5	0.1	0.8	1.1	2.6	1.4	3.6	2.9	2.5	9.4	0.4	0.0	0.7	4.7	0.0	1.3	0.8	1.1
LAMQNE (n=1)	7.6	0.4	0.0	2.7	25.4	30.7	0.7	4.7	1.4	0.2	0.7	0.6	0.5	0.2	3.0	1.5	3.6	7.8	0.3	0.0	1.4	3.7	0.0	1.7	0.8	0.3
LAMQSF (n=1)	5.4	0.3	0.0	3.8	20.3	30.2	0.5	3.9	3.6	0.5	0.8	0.6	0.4	0.1	1.3	0.9	6.1	13.3	0.3	0.0	1.0	4.0	0.0	2.4	0.0	0.0
LAMRCO (n=1)	6.7	0.4	0.0	4.2	14.8	26.6	1.0	2.4	4.7	0.5	1.0	0.4	0.3	0.0	3.9	1.5	6.7	12.3	0.4	0.0	2.6	5.3	0.0	3.5	0.5	0.0
LAMRDS (n=1)	4.9	0.0	0.0	3.4	20.9	31.3	1.4	2.7	2.7	0.2	0.9	0.6	0.3	6.6	0.0	1.2	4.0	9.1	0.3	0.0	2.7	3.8	0.0	2.1	0.7	0.0
LAMRHL (n=1)	6.4	0.3	0.0	4.0	19.7	23.4	0.8	2.6	1.1	0.2	1.0	0.7	1.9	0.5	4.4	2.7	4.7	13.7	0.3	0.0	1.3	5.0	0.0	2.8	0.6	1.8
LAMRJK (n=1)	5.0	0.2	0.0	3.6	25.5	31.1	1.5	4.3	2.6	0.4	0.9	0.7	0.0	0.0	2.8	1.4	4.0	9.5	0.0	0.0	1.3	3.8	0.0	1.7	0.0	
LAMRNQ (n=1)	5.0	0.2	0.1	3.1	23.5	31.7	0.6	4.2	1.2	0.2	0.8	0.8	1.4	0.4	3.4	1.8	5.5	8.5	0.3	0.0	1.1	3.0	0.0	1.8	0.5	0.9
LAMVUB (n=1)	4.3	0.2	0.0	3.2	21.9	33.5	0.2	4.8	0.4	0.1	0.8	1.0	3.0	1.2	3.7	2.4	4.7	8.4	0.3	0.0	0.6	3.0	0.0	1.7	0.6	
LAMYDP (n=1)	3.4	0.1	0.0	2.9	19.2	29.9	1.1	4.6	2.8	0.4	0.7	0.7	0.4	0.1	2.0	1.5	4.2	14.1	0.3	0.0	1.9	5.7	0.0	3.3	0.5	0.2
LANBCH (n=1)	3.7	0.1	0.0	3.2	19.5	31.2	1.0	4.7	2.2	0.4	0.8	0.7	0.3	0.0	1.9	1.6	4.5	12.5	0.3	0.0	2.1	5.7	0.0	3.2	0.0	0.3
LANCEG (n=1)	5.0	0.0	0.0	3.7	22.4	33.6	0.8	4.1	2.0	0.3	0.9	0.6	0.1	5.5	0.4	0.7	1.3	11.2	0.2	0.0	1.2	4.0	0.0	2.0	0.0	0.0
LANCOX (n=1)	4.9	0.2	0.0	3.2	22.1	33.9	0.6	4.4	2.3	0.4	0.8	0.6	0.0	4.9	0.3	0.8	1.6	10.5	0.3	0.0	1.4	4.1	0.0	2.6	0.0	0.0
LANFEF (n=1)	5.3	0.0	0.0	2.5	28.8	32.0	0.0	6.0	0.3	0.0	0.6	1.0	2.7	1.1	2.7	1.6	2.4	6.3	0.0	0.0	1.0	2.9	0.0	1.1	0.9	0.8
LANMGC (n=1)	5.2	0.2	0.0	3.4	25.2	33.1	1.0	4.1	2.2	0.2	0.8	0.6	0.2	0.0	5.1	2.3	2.7	6.5	0.3	0.0	1.1	2.6	0.0	1.4	1.3	0.0
LANPMZ (n=1)	3.6	0.1	0.0	3.1	22.5	32.7	0.3	5.8	0.4	0.1	0.8	1.0	2.0	0.8	3.0	2.6	2.7	8.8	0.4	0.0	1.2	4.1	0.0	2.2	0.9	1.0
LANMOM (n=1)	5.3	0.2	0.0	2.8	19.6	32.5	0.8	4.5	2.2	0.4	0.7	0.6	0.4	0.2	2.3	1.6	4.3	11.9	0.3	0.0	1.5	4.6	0.0	2.5	0.4	0.3
LANTLE (n=1)	4.4	0.3	0.0	2.3	19.0	31.5	0.5	5.3	1.6	0.3	0.6	0.7	1.4	0.7	3.2	2.5	5.1	11.6	0.2	0.0	1.0	4.7	0.0	2.6	0.2	0.4
LANTSP (n=1)	5.3	0.2	0.0	2.9	23.5	32.1	0.8	4.6	1.9	0.3	0.7	0.7	0.4	0.1	2.3	1.5	3.6	9.9	0.3	0.0	1.8	3.9	0.0	2.3	0.6	0.1
LANUCB (n=1)	4.8	0.3	0.0	3.4	25.7	27.9	2.1	3.6	4.2	0.6	0.8	0.7	0.1	0.0	1.9	1.1	4.2	10.7	0.3	0.0	1.3	4.0	0.0	1.9	0.0	0.3
LAOBGQ (n=1)	4.8	0.2	0.0	3.4	23.0	34.0	0.9	3.9	2.0	0.2	0.9	0.7	0.4	0.0	2.4	1.2	4.8	9.2	0.4	0.0	1.6	3.8	0.0	1.9	0.0	0.2
LAOHLR (n=1)	5.5	0.2	0.0	3.2	21.7	34.5	1.1	3.2	2.3	0.2	0.8	0.6	0.0	0.0	4.6	1.6	6.0	8.4	0.3	0.0	1.1	2.6	0.0	1.6	0.3	0.0
LAOJAT (n=1)	5.3	0.2	0.0	2.9	22.5	34.1	0.8	5.0	2.3	0.4	0.6	0.6	0.4	0.0	2.1	1.3	4.3	10.3	0.0	0.0	1.0	3.6	0.0	2.0	0.0	0.2
LAOKLP (n=1)	5.0	0.2	0.0	3.1	22.9	32.5	0.2	5.5	0.4	0.1	0.8	1.0	3.0	1.3	3.2	2.5	3.4	8.4	0.3	0.0	0.7	3.3	0.0	1.8	0.6	0.0
LAOKTE (n=1)	4.9	0.2	0.0	2.6	21.3	29.8	0.9	4.6	1.9	0.3	0.7	0.6	0.3	0.2	2.3	1.6	5.4	12.6	0.3	0.0	1.4	4.8	0.0	2.4	0.4	0.3
LAPKLS (n=1)	5.7	0.3	0.0	3.8	20.0	32.5	1.1	3.9	3.0	0.4	0.8	0.6	0.0	0.0	3.1	2.0	4.0	10.6	0.2	0.0	1.4	3.5	0.0	2.3	0.6	0.1
LAPKXM (n=1)	4.4	0.2	0.0	3.6	23.0	33.1	0.8	4.3	1.7	0.2	0.9	0.7	0.5	0.0	2.3	1.6	4.3	13.3	0.4	0.0	0.6	2.5	0.0	1.2	0.0	0.3
LAPKZJ (n=1)	4.8	0.1	0.0	3.7	23.2	30.4	0.4	4.7	0.6	0.1	0.9	1.0	2.2	0.9	3.4	2.7	2.9	9.2	0.4	0.0	0.9	3.7	0.0	1.9	0.8	1.1
LAPWJD (n=1)	5.5	0.1	0.0	3.5	25.6	35.2	0.4	4.6	0.7	0.0	0.8	0.8	1.5	0.6	3.4	2.1	2.7	6.6	0.2	0.0	0.8	2.4	0.0	1.4	0.6	0.7
LAPWLP (n=1)	4.5	0.1	0.0	2.9	28.2	18.2	0.5	4.7	0.5	0.2	0.8	1.2	2.9	1.1	1.9	2.1	3.6	15.9	0.4	0.0	0.7	4.8	0.0	3.2	0.0	1.7
LAQYTA (n=1)	5.3	0.0	0.0	3.0	24.8	30.1	1.2	4.3	2.1	0.1	0.7	0.6	0.4	0.0	2.1	1.1	7.5	10.2	0.0	0.0	0.9	3.7	0.0	1.5	0.0	0.5
LAQYUT (n=1)	5.6	0.2	0.0	3.5	21.5	32.5	1.4	3.0	3.1	0.3	0.7	0.6	0.0	0.0	4.6	1.6	6.8	8.9	0.0	0.0	1.0	2.6	0.0	1.6	0.0	0.3
LAQYWQ (n=1)	5.8	0.3	0.0	4.3	22.3	30.0	2.1	3.6	4.1	0.6	0.8	0.5	0.3	0.0	1.7	1.1	3.8	11.5	0.0	0.0	1.5	3.5	0.0	1.9	0.0	0.1
LAQZME (n=1)	6.3	0.3	0.0	2.5	21.7	30.7	0.7	3.3	2.7	0.4	0.6	0.6	0.7	0.2	2.7	1.6	6.4	10.3	0.3	0.0	1.2	3.8	0.0	2.6	0.1	0.2

Table 44: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=296)	4.9 ± 0.5	0.2 ± 0.1	0 ± 0	3.2 ± 0.6	28.5 ± 5.4	31 ± 4	0.5 ± 0.3	4.7 ± 1	0.8 ± 0.5	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.2	1.6 ± 0.9	0.7 ± 1.2	2.7 ± 0.9	1.6 ± 0.6	4.6 ± 2.3	7.2 ± 1.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.3	2.7 ± 0.7	0 ± 0	1.3 ± 0.4	0.3 ± 0.3	0.9 ± 0.5
dc (n=198)	5.1 ± 0.6	0.2 ± 0.1	0 ± 0	3.5 ± 0.6	23.7 ± 3.1	32.2 ± 2.5	1.1 ± 0.3	4.1 ± 0.7	2.3 ± 0.6	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.3 ± 0.2	0.4 ± 1.4	3 ± 2	1.6 ± 0.8	4 ± 1.8	9.1 ± 2.8	0.3 ± 0.1	0 ± 0	1.4 ± 0.5	3.4 ± 1.2	0 ± 0	1.8 ± 0.6	0.6 ± 0.7	0.3 ± 0.2
tc (n=2)	5.6 ± 0.4	0.3 ± 0	0 ± 0	4.4 ± 0.7	22 ± 1.5	30.2 ± 0.1	1.6 ± 0.7	3.6 ± 1.2	3.1 ± 0.7	0.4 ± 0.1	0.9 ± 0	0.6 ± 0	0.3 ± 0.1	0.1 ± 0.1	3.5 ± 1.5	1.7 ± 0.4	3.4 ± 1.1	9.7 ± 3	0.3 ± 0	0 ± 0	1.6 ± 0.2	3.6 ± 1.4	0 ± 0	2.2 ± 0.7	0.7 ± 0	

Table 45: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
sc (n=1)	5.3	0.3	0.0	3.2	26.0	32.3	0.6	4.5	1.0	0.2	0.8	0.8	1.1	0.4	1.8	1.1	5.7	9.3	0.4	0.0	0.7	3.2	0.0	1.4	0.0	0.0
dc (n=1)	4.5	0.1	0.0	2.9	28.2	18.2	0.5	4.7	0.5	0.2	0.8	1.2	2.9	1.1	1.9	2.1	3.6	15.9	0.4	0.0	0.7	4.8	0.0	3.2	0.0	1.7
tc (n=1)	3.5	0.1	0.0	2.7	18.2	26.4	0.4	4.6	0.9	0.2	0.7	0.9	2.0	0.7	2.2	1.9	5.5	16.7	0.3	0.0	1.2	6.6	0.0	3.3	0.2	1.0

Table 46: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF:

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deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
LALHCY (n=15)	59.3 ± 2.6	1 ± 0	1 ± 0	1 ± 0	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.1	4 ± 0	136 ± 3.9	4.7 ± 0.2	3.3 ± 1.1
LALJAO (n=15)	65.9 ± 3.2	1 ± 0	1.2 ± 0.8	1.3 ± 0.5	1.8 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.3 ± 1.3	3.5 ± 0.9	124.3 ± 3.7	4 ± 0.5	3 ± 0.7
LALJKA (n=15)	62.1 ± 5.5	1 ± 0	1 ± 0	1.1 ± 0.3	2.5 ± 2.2	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.8	3.6 ± 0.7	126 ± 5.1	4.6 ± 0.2	3.4 ± 2
LALLTL (n=11)	55.3 ± 2.2	1 ± 0	1 ± 0	1 ± 0	1.5 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	128.6 ± 5.5	4.5 ± 0.2	2.2 ± 1.2
LALQAM (n=15)	58.3 ± 2.8	1 ± 0	1 ± 0	1 ± 0	2.7 ± 2.1	3 ± 0	3 ± 0	5 ± 0	2.1 ± 2.3	4 ± 0	133.7 ± 4	4.8 ± 0.2	4.7 ± 1.8
LALQDS (n=14)	54.5 ± 3.1	1 ± 0	1 ± 0	1.6 ± 0.9	2.6 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.9 ± 1.6	4 ± 0	127.1 ± 5.4	4.6 ± 0.2	3.4 ± 1.4
LALRCQ (n=14)	61.7 ± 1.7	1 ± 0	1 ± 0	1 ± 0	2.1 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.9 ± 1.5	3.7 ± 0.7	127.1 ± 3.8	4.8 ± 0.3	3.3 ± 1.2
LALWKF (n=14)	62.1 ± 3.2	1 ± 0	1.4 ± 1.1	1 ± 0	1.4 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	132.9 ± 5.8	4.5 ± 0.2	3.1 ± 0.8
LAMABL (n=8)	56.1 ± 1.6	1 ± 0	2.9 ± 1.6	2.1 ± 0.6	6.5 ± 2	3 ± 0	3 ± 0	5 ± 0	5 ± 2.3	4 ± 0	123.1 ± 4.6	4.1 ± 0.4	5.3 ± 1.6
LAMCKI (n=10)	61.5 ± 2.4	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	131 ± 3.9	4.1 ± 0.2	2.8 ± 0.6
LAMCLE (n=14)	65.4 ± 1.9	1 ± 0	1.4 ± 1.1	1.5 ± 0.8	2 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.6	3.7 ± 0.7	124.3 ± 4.3	4.3 ± 0.3	3 ± 1
LAMCLF (n=9)	66.4 ± 4.3	1 ± 0	1.3 ± 1	1 ± 0	1.1 ± 0.3	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.1 ± 1.1	121.1 ± 3.3	4 ± 0.5	2.1 ± 0.3
LAMEUB (n=15)	57.7 ± 2.4	1 ± 0	1 ± 0	1.5 ± 0.7	4.5 ± 2.3	3 ± 0	3 ± 0	5 ± 0	3.9 ± 2.3	4 ± 0	128.3 ± 3.6	4.2 ± 0.4	3.1 ± 1
LAMEUU (n=13)	65.8 ± 3.6	1 ± 0	1.5 ± 1.1	1.5 ± 0.9	2.2 ± 1.9	3 ± 0	3 ± 0	5 ± 0	2.1 ± 1.7	3.7 ± 0.8	126.2 ± 4.2	4.2 ± 0.4	3.2 ± 0.7
LAMFJO (n=15)	64.7 ± 2.9	1 ± 0	1.6 ± 1.2	1.1 ± 0.3	3.7 ± 2.4	3 ± 0	3 ± 0	5 ± 0	2.3 ± 1.7	4 ± 0	123.7 ± 3.5	4.1 ± 0.5	4.1 ± 2.4
LAMIRY (n=10)	54.7 ± 2.3	1 ± 0	1 ± 0	1 ± 0	2.2 ± 2.1	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.9	4 ± 0	123 ± 4.2	4.9 ± 0.2	3.3 ± 1.7
LAMJIC (n=9)	60.1 ± 2.9	1 ± 0	2 ± 1.5	2.7 ± 2.5	5.3 ± 3.1	3 ± 0	3 ± 0	5 ± 0	3.6 ± 2	3.3 ± 1	111.1 ± 26.8	3.9 ± 0.7	4.4 ± 1.1
LAMPJB (n=13)	64.8 ± 2.5	1 ± 0	1.1 ± 0.3	1 ± 0	2.1 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.5 ± 0.9	4 ± 0	123.5 ± 2.4	4.6 ± 0.3	3.8 ± 1.5
LAMQDL (n=14)	67.9 ± 4.5	1 ± 0	1.2 ± 0.8	1.6 ± 0.8	2.6 ± 2.3	3 ± 0	3 ± 0	5 ± 0	1.6 ± 0.9	4 ± 0	117.1 ± 6.4	3.5 ± 0.4	3.8 ± 1.6
LAMQNE (n=14)	71.9 ± 6.2	1 ± 0	1.2 ± 0.8	1 ± 0	1.5 ± 1.3	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	119.3 ± 3.3	4.2 ± 0.3	3.6 ± 1.3
LAMQSF (n=14)	61.1 ± 1.8	1 ± 0	1 ± 0	1 ± 0	1.6 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	123.9 ± 4	3.9 ± 0.5	2.6 ± 0.7
LAMRCO (n=14)	62.1 ± 2.9	1 ± 0	1 ± 0	1.6 ± 1	2.9 ± 1.7	3 ± 0	3 ± 0	5 ± 0	2.1 ± 1.3	4 ± 0	119.3 ± 4.3	3.9 ± 0.4	3.9 ± 2.1
LAMRDS (n=15)	63.7 ± 2.3	1 ± 0	1.4 ± 1.1	1.1 ± 0.3	1.9 ± 1.4	3 ± 0	3 ± 0	5 ± 0	1.4 ± 1.1	4 ± 0	125.7 ± 3.7	4.4 ± 0.4	2.7 ± 1.3
LAMRHL (n=14)	67.7 ± 4	1 ± 0	1 ± 0	1 ± 0	2.9 ± 2	3 ± 0	3 ± 0	5 ± 0	2.7 ± 2.4	4 ± 0	122.1 ± 2.6	4.1 ± 0.5	4.1 ± 1.1
LAMRJK (n=15)	63.4 ± 2.6	1 ± 0	1.3 ± 1	1 ± 0	3.5 ± 2	3 ± 0	3 ± 0	5 ± 0	2.5 ± 1.6	4 ± 0	124.7 ± 4.4	4.5 ± 0.4	3.8 ± 1.1
LAMRNQ (n=9)	63.1 ± 3	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	121.9 ± 2.6	3.8 ± 0.5	2.6 ± 1.3
LAMVUB (n=15)	65.5 ± 3.3	1 ± 0	1 ± 0	1 ± 0	3.3 ± 2.6	3 ± 0	3 ± 0	5 ± 0	2.3 ± 1.9	4 ± 0	124.3 ± 4.2	4.6 ± 0.3	4.2 ± 2.2
LAMYDP (n=14)	64.1 ± 4.6	1 ± 0	1 ± 0	1 ± 0	1.9 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.4 ± 0.9	4 ± 0	123.2 ± 3.7	4.5 ± 0.3	3.9 ± 1.4
LANBCH (n=14)	64.4 ± 2.8	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	123.9 ± 3.5	4.2 ± 0.2	3.1 ± 0.7

LANCEG (n=4)	67.3 ± 3.3	1 ± 0	1 ± 0	1.3 ± 0.5	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	123.8 ± 2.5	4.1 ± 0.4	2.5 ± 0.6
LANCOX (n=10)	66.3 ± 3.2	1 ± 0	1 ± 0	1.6 ± 0.8	4.2 ± 2.3	3 ± 0	3 ± 0	5 ± 0	2.9 ± 1.4	3.6 ± 0.8	127.5 ± 2.6	4.6 ± 0.3	4 ± 1.5
LANFEF (n=15)	67.4 ± 2.4	1 ± 0	1.2 ± 0.8	1.1 ± 0.4	1.5 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.5	4 ± 0	127 ± 3.2	4.7 ± 0.3	2.9 ± 0.5
LANMGC (n=15)	64.2 ± 2	1 ± 0	1 ± 0	1 ± 0	2.3 ± 2.2	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.6	4 ± 0	123.7 ± 4	4.4 ± 0.3	3 ± 0.8
LANMOM (n=10)	65.9 ± 3.1	1 ± 0	1 ± 0	1 ± 0	1.4 ± 1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.3	4 ± 0	119.5 ± 3.7	3.4 ± 0.7	3.3 ± 1.2
LANPMZ (n=13)	65.3 ± 3.1	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	124.2 ± 3.4	4.4 ± 0.2	2.4 ± 0.5
LANTLE (n=15)	55.6 ± 2	1 ± 0	2.5 ± 1.7	3.1 ± 0.9	1.9 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.6	3.9 ± 0.5	106 ± 9.3	3.2 ± 0.3	2.6 ± 0.8
LANTSP (n=12)	65.3 ± 3.4	1 ± 0	1 ± 0	1.2 ± 0.4	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	124.6 ± 4	4.1 ± 0.4	3.8 ± 1.3
LANUCB (n=14)	58.3 ± 2.2	1 ± 0	1.2 ± 0.8	2.6 ± 0.6	1.9 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.4 ± 0.7	4 ± 0	117.9 ± 5.4	3.6 ± 0.3	3.1 ± 1
LAOBGQ (n=15)	67.8 ± 3.4	1 ± 0	1 ± 0	1.1 ± 0.4	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	127 ± 3.2	4.2 ± 0.3	3.1 ± 0.6
LAOHLR (n=14)	61.7 ± 2.7	1 ± 0	1 ± 0	1 ± 0	1.1 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	123.2 ± 4.2	4.2 ± 0.5	2.8 ± 0.7
LAOJAT (n=15)	60.3 ± 2.8	1 ± 0	1 ± 0	1.4 ± 0.6	1.4 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	124.3 ± 5	4 ± 0.4	4.8 ± 1.6
LAOKLP (n=15)	62 ± 2.9	1 ± 0	1 ± 0	1.1 ± 0.4	1.9 ± 1.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125.7 ± 3.2	4.1 ± 0.3	3.8 ± 1.9
LAOKTE (n=15)	62.9 ± 3.7	1 ± 0	1.4 ± 1.1	1.7 ± 0.6	2.1 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 1	4 ± 0	122.3 ± 4.2	3.7 ± 0.5	5.6 ± 2.7
LAPKLS (n=12)	66.3 ± 2.7	1 ± 0	1 ± 0	1.7 ± 0.9	1.9 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	2.8 ± 1	118.3 ± 6.2	4.2 ± 0.3	4.5 ± 1.4
LAPKXM (n=15)	68.2 ± 3.2	1 ± 0	1.2 ± 0.8	1.9 ± 0.7	1.3 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.2 ± 1	127.3 ± 2.6	4 ± 0.4	3.5 ± 0.5
LAPKZJ (n=15)	67.9 ± 2.7	1 ± 0	1.2 ± 0.8	1.1 ± 0.3	1.5 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	127 ± 3.2	4.2 ± 0.3	3.3 ± 0.8
LAPWJD (n=14)	68.3 ± 2.9	1 ± 0	1 ± 0	1.1 ± 0.4	1.7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.1 ± 4	4.6 ± 0.3	3.1 ± 0.3
LAPWLP (n=14)	64.9 ± 4	1 ± 0	1.5 ± 1.3	2 ± 1.4	3.6 ± 3	3 ± 0	3 ± 0	5 ± 0	2.6 ± 2.8	3.6 ± 0.9	119.3 ± 8.7	3.5 ± 0.4	4.1 ± 0.9
LAQYTA (n=15)	59.2 ± 3.9	1 ± 0	1.2 ± 0.8	2.6 ± 0.8	2.1 ± 1.5	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1	3.7 ± 0.7	111.3 ± 6.1	3.6 ± 0.3	4.2 ± 1.3
LAQYUT (n=15)	66.3 ± 3	1 ± 0	1 ± 0	1.1 ± 0.3	1.6 ± 0.5	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.7 ± 0.7	126.3 ± 3	4.3 ± 0.2	3.7 ± 0.6
LAQYWQ (n=15)	66.7 ± 4.6	1 ± 0	1 ± 0	1.6 ± 0.7	2.5 ± 1.8	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	4 ± 0	124 ± 3.9	4.1 ± 0.4	3.8 ± 0.7
LAQZME (n=12)	64.3 ± 4.7	3 ± 3.6	1.8 ± 1.5	1.5 ± 0.5	4.1 ± 3.4	3 ± 0	3 ± 0	5 ± 0	2.8 ± 3.3	4 ± 0	124.2 ± 5.1	4.2 ± 0.4	4.8 ± 2.8

Table 47: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 41. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=296)	62.9 ± 4.8	1 ± 0	1.2 ± 0.7	1.4 ± 0.9	2.5 ± 2	3 ± 0	3 ± 0	5 ± 0	1.7 ± 1.6	3.9 ± 0.4	123.9 ± 8	4.2 ± 0.6	3.6 ± 1.6

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dc (n=198)	63.4 ± 4.5	1 ± 0.6	1.2 ± 0.7	1.2 ± 0.5	2 ± 1.6	3 ± 0	3 ± 0	5 ± 0	1.6 ± 1.4	3.8 ± 0.5	123.9 ± 10.4	4.2 ± 0.4	3.6 ± 1.6
tc (n=2)	59.5 ± 0.7	1 ± 0	1 ± 0	1 ± 0	1 ± 0	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125 ± 0	3.7 ± 0.1	3.5 ± 0.7

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during summer

Table 48 shows the copy number analysis of select events. The events comprised one to two homozygous insertions and some had additional insertions still segregating. For example LANBCH segregated as homozygous for one T-DNA insertions for each construct, while LANPMZ segregated as homozygous for two T-DNA insertions for each construct. LALXOL seems to segregate for one insertion of VC-LLM337-1qcz rc, not homozygous, and for one homozygous insertion of LJB2197-1qcz_F with another copy which was not homozygous with the exception of the region around j-t-StCAT_p2_p-LuPXR, which seems to be a double copy event homozygous for each copy. For the T2 events selected, combined DHA and EPA levels were from nine to thirteen percent of the total fatty acids present in the seed. Whereas the selected T3 events had combined DHA and EPA levels varying from eleven to twenty three percent, with LALWPA having a DHA level of five percent and an EPA level of eighteen percent with respect to total fatty acid content in the seed, see Table 50. The selected events exhibited no morphological or anatomical defects relative to one another or to wild type.

Table 48: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. As the T2 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative of one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.							Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.							
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnI-2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LANBCH (n=30)	4.0	3.7	4.1	4.0	4.4	4.0	3.8	2.9	3.9				3.8	3.8	3.8
LANPMZ (n=74)	2.0	2.0	2.0	2.0	1.6	2.0	2.0	1.8	2.1				2.0	2.0	2.1
LAOIKC (n=29)	2.1	4.0	3.9	4.2	4.3	4.1	4.3	2.3	2.0				2.1	2.3	2.2
LALHBO (n=29)	4.1	3.9	4.1	4.0	3.9	3.9	3.8	3.9	3.5				3.8	2.1	2.0
LALRCH (n=30)	4.1	3.8	4.0	3.9	3.4	4.1	4.0	3.9	4.1				3.6	3.8	4.0
LALWPA (n=29)	4.0	3.9	4.1	6.0	5.7	4.1	3.9	2.3	3.6				3.5	3.4	3.6
LALXOL (n=15)	2.1	2.1	2.1	4.1	2.7	2.0	2.1	0.9	1.2				1.2	1.1	1.2
LALXVM (n=30)	3.8	3.6	3.9	3.5	1.8	2.0	1.9	2.5	2.5				2.4	2.2	2.3
LALZGS (n=24)	1.9	4.0	3.8	4.0	1.8	2.0	2.0	2.1	2.0				2.0	2.1	2.2
LAMADR (n=44)	4.0	3.9	3.8	4.0	4.6	4.0	3.9	2.5	3.4				3.1	3.0	3.1
LAMQJH (n=30)	4.0	4.0	4.0	3.9	2.9	4.1	4.1	4.5	4.1				4.0	3.9	4.2
LAMQUI (n=36)	2.2	2.1	4.0	3.7	3.5	3.6	3.8	1.5	1.3				2.9	5.2	5.5
LAMRUR (n=30)	3.6	3.7	3.4	3.7	4.1	3.6	3.8	3.7	3.6				3.8	4.3	3.9

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LANPSF (n=30)	4.0	4.0	3.8	3.9	4.1	3.9	4.1	1.5	3.5				3.4	4.3	3.9										

Table 49: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T3 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANBCH (n=30)	4.5 ± 0.6	0.2 ± 0	0.1 ± 0	4 ± 0.2	22.2 ± 1.1	31.1 ± 0.7	1 ± 0.1	4 ± 0.2	1.8 ± 0.2	0.2 ± 0	0.8 ± 0	0.6 ± 0	0.5 ± 0.1	0.1 ± 0	2.6 ± 0.3	1.8 ± 0.1	3.3 ± 0.1	11.4 ± 0.5	0.3 ± 0	0 ± 0	1.7 ± 0.1	4.4 ± 0.2	0 ± 0	2.3 ± 0.2	0.6 ± 0.1	0.4 ± 0.1
LANPMZ (n=74)	5 ± 0.4	0.2 ± 0	0 ± 0	3.6 ± 0.4	26.3 ± 2.1	35.2 ± 1.6	0.4 ± 0.1	4.6 ± 0.6	0.7 ± 0.2	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.1 ± 0.4	0.3 ± 0.2	2.4 ± 0.3	1.5 ± 0.2	3.1 ± 0.4	7.2 ± 0.8	0.3 ± 0.1	0 ± 0	1 ± 0.1	2.9 ± 0.5	0 ± 0	1.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.1
LAOIKC (n=29)	5.1 ± 0.6	0.2 ± 0	0.1 ± 0	3.6 ± 0.3	22.1 ± 1.7	30.8 ± 1	0.9 ± 0.1	4 ± 0.3	1.8 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.6 ± 0	0.5 ± 0.1	0.1 ± 0	3.1 ± 0.8	1.6 ± 0.3	5.9 ± 0.5	10.3 ± 0.8	0.3 ± 0	0 ± 0	1.3 ± 0.1	4 ± 0.3	0 ± 0	1.8 ± 0.2	0.4 ± 0.1	0.3 ± 0
LALHBO (n=29)	4.4 ± 0.3	0.2 ± 0.1	0 ± 0	3.9 ± 0.4	19.6 ± 2.3	28.8 ± 1.5	0.6 ± 0.1	4.9 ± 0.3	1.3 ± 0.3	0.2 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.9 ± 0.3	0.3 ± 0.1	2.4 ± 0.3	2 ± 0.2	4.3 ± 0.3	13.4 ± 1.9	0.3 ± 0	0 ± 0	1.3 ± 0.2	5.7 ± 0.8	0 ± 0	2.7 ± 0.4	0.4 ± 0.1	0.6 ± 0.1
LALRCH (n=30)	4.6 ± 0.2	0.2 ± 0	0.1 ± 0	3.7 ± 0.3	22.3 ± 1.2	30.1 ± 1	0.9 ± 0.1	4.2 ± 0.2	1.6 ± 0.2	0.2 ± 0	0.8 ± 0	0.7 ± 0	0.7 ± 0.2	0.2 ± 0.1	2.7 ± 0.3	1.8 ± 0.2	4 ± 0.4	11.2 ± 0.8	0.3 ± 0	0 ± 0	1.8 ± 0.1	4.5 ± 0.4	0 ± 0	2.3 ± 0.4	0.6 ± 0.1	0.5 ± 0.2
LALWPA (n=29)	4.4 ± 0.2	0.2 ± 0.1	0 ± 0	3.3 ± 0.2	17.3 ± 1.6	25.6 ± 1.4	1 ± 0.1	4.4 ± 0.4	2.3 ± 0.3	0.4 ± 0.1	0.8 ± 0	0.7 ± 0	0.5 ± 0.2	0.2 ± 0	2 ± 0.2	1.6 ± 0.1	5.7 ± 0.7	14.8 ± 1.5	0.4 ± 0	0 ± 0	2.1 ± 0.2	7.3 ± 0.8	0 ± 0	4 ± 0.7	0.5 ± 0.1	0.3 ± 0.1
LALXOL (n=15)	4.4 ± 0.3	0.2 ± 0	0 ± 0	4.4 ± 0.6	21.4 ± 1.1	28.4 ± 1.2	0.4 ± 0.1	3.5 ± 0.3	0.8 ± 0.2	0.1 ± 0	0.9 ± 0.1	0.9 ± 0.1	2.5 ± 0.5	0.5 ± 0.2	2.2 ± 0.3	1.3 ± 0.3	8.7 ± 2.1	11.6 ± 1.3	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.5 ± 0.8	0 ± 0	1.8 ± 0.4	0.1 ± 0.1	1 ± 0.2
LALXVM (n=30)	4.5 ± 1	0.2 ± 0	0 ± 0	3.8 ± 0.4	21.3 ± 1.6	28.8 ± 1.7	0.7 ± 0.2	4.5 ± 0.6	1.2 ± 0.6	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	1.5 ± 0.8	0.5 ± 0.3	2.7 ± 0.3	1.9 ± 0.3	4.7 ± 0.9	11.7 ± 1.3	0.3 ± 0	0 ± 0	1.4 ± 0.4	5.1 ± 0.8	0 ± 0	2.3 ± 0.3	0.4 ± 0.2	0.8 ± 0.4
LALZGS (n=24)	4.5 ± 0.7	0.3 ± 0	0 ± 0	3.4 ± 0.5	21.8 ± 1.8	29.4 ± 1.4	0.6 ± 0.1	4.2 ± 0.5	1.2 ± 0.3	0.2 ± 0	0.8 ± 0.1	0.7 ± 0.1	1.3 ± 0.3	0.3 ± 0.1	2.6 ± 0.3	1.8 ± 0.3	5.6 ± 0.6	12.1 ± 1.2	0.3 ± 0	0 ± 0	1.2 ± 0.1	4.7 ± 0.6	0 ± 0	2.1 ± 0.4	0.2 ± 0.2	0.8 ± 0.2
LAMADR (n=44)	3.7 ± 1.6	0.2 ± 0	0.1 ± 0	4 ± 0.5	22.3 ± 2.8	32.5 ± 1.5	0.8 ± 0.2	4.3 ± 0.6	1.9 ± 0.4	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.5 ± 0.2	0.1 ± 0	2.2 ± 0.4	1.4 ± 0.2	4.4 ± 2	10.8 ± 1.6	0.3 ± 0	0 ± 0	1.6 ± 0.2	4.1 ± 0.5	0 ± 0	2.1 ± 0.3	0.5 ± 0.2	0.3 ± 0.1
LAMQJH (n=30)	4.9 ± 0.3	0.3 ± 0	0.1 ± 0.1	3.8 ± 0.3	21.9 ± 1.7	30.9 ± 1.1	1 ± 0.2	4.3 ± 0.3	2.2 ± 0.2	0.3 ± 0.1	0.8 ± 0	0.6 ± 0	0.2 ± 0.1	0 ± 0	3 ± 0.5	1.7 ± 0.2	3.4 ± 0.4	10.7 ± 1	0.3 ± 0.1	0 ± 0	1.8 ± 0.2	4.4 ± 0.6	0 ± 0	2.3 ± 0.5	0.7 ± 0.1	0.2 ± 0
LAMQUI (n=36)	3.2 ± 2	0.2 ± 0.1	0.1 ± 0	3.1 ± 0.8	25.1 ± 2.7	26 ± 1.4	1.1 ± 0.3	4.5 ± 0.9	1.5 ± 0.4	0.2 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.1 ± 0.7	0.3 ± 0.2	2.2 ± 0.4	1.3 ± 0.3	4.7 ± 1.7	11.6 ± 2.2	0.2 ± 0	0 ± 0	2.4 ± 0.4	6.1 ± 0.9	0 ± 0	2.3 ± 0.6	0.6 ± 0.1	0.7 ± 0.3

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LAMRUR (n=30)	4.9 ± 0.3	0.2 ± 0	0.2 ± 0.1	3.9 ± 0.3	23.4 ± 1.7	32 ± 1.1	1.1 ± 0.2	4 ± 0.5	2.3 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.2 ± 0.1	0 ± 0	2.9 ± 0.6	1.5 ± 0.2	4 ± 0.4	9.4 ± 0.9	0.2 ± 0.1	0 ± 0	1.6 ± 0.2	3.7 ± 0.3	0 ± 0	1.9 ± 0.3	0.6 ± 0.1	0.2 ± 0.1
LANPSF (n=30)	4.7 ± 0.2	0.3 ± 0	0.1 ± 0	3.9 ± 0.2	23.2 ± 1.3	30.9 ± 0.9	0.8 ± 0.1	4.5 ± 0.5	1.7 ± 0.3	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.5 ± 0.1	0.1 ± 0	2.2 ± 0.2	1.5 ± 0.1	3.7 ± 0.7	9.9 ± 0.8	0.3 ± 0	0 ± 0	2.1 ± 0.2	4.4 ± 0.4	0 ± 0	2.3 ± 0.2	0.7 ± 0.2	0.3 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 50: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANBCH (n=1)	4.6	0.2	0.1	3.9	21.3	30.3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	12.4	0.3	0.0	1.8	4.8	0.0	2.5	0.7	0.3
LANPMZ (n=1)	4.6	0.3	0.1	4.0	22.6	32.2	0.3	5.2	0.7	0.1	0.9	0.8	1.4	0.5	2.6	2.1	3.1	9.7	0.3	0.0	1.1	4.2	0.0	2.3	0.5	0.5
LAOIKC (n=1)	7.9	0.5	0.1	3.2	19.0	28.4	0.8	3.4	1.9	0.2	0.7	0.5	0.5	0.1	3.0	1.6	6.5	12.2	0.3	0.0	1.5	4.6	0.0	2.2	0.3	0.4
LALHBO (n=1)	4.1	0.1	0.0	3.6	16.4	26.5	0.6	4.8	1.5	0.3	0.9	0.7	1.1	0.4	2.7	2.3	4.3	16.6	0.4	0.0	1.4	7.0	0.0	3.5	0.5	0.6
LALRCH (n=1)	4.1	0.2	0.1	3.6	20.5	28.5	0.8	4.3	1.3	0.2	0.7	0.7	1.2	0.3	2.7	2.0	4.6	12.3	0.3	0.0	1.8	5.0	0.0	3.3	0.6	1.0
LALWPA (n=1)	4.7	0.2	0.0	3.0	14.6	20.2	1.1	4.7	2.8	0.7	0.8	0.7	0.7	0.2	1.6	1.6	5.4	18.1	0.4	0.0	2.0	10.0	0.0	5.7	0.5	0.4
LALXOL (n=1)	4.6	0.2	0.0	4.9	21.0	26.0	0.5	4.2	0.7	0.2	1.0	0.9	2.3	0.9	2.1	1.9	3.4	14.0	0.4	0.0	1.1	5.4	0.0	2.7	0.4	1.1
LALXVM (n=1)	4.7	0.3	0.0	3.7	18.3	26.9	0.5	4.5	0.8	0.2	0.8	0.9	2.4	0.8	2.7	2.2	4.7	14.3	0.3	0.0	1.1	5.6	0.0	2.8	0.4	1.1
LALZGS (n=1)	4.6	0.3	0.0	2.5	19.0	28.4	0.6	3.7	1.5	0.2	0.6	0.7	1.2	0.3	2.7	1.9	7.0	14.1	0.2	0.0	1.4	5.5	0.0	2.9	0.0	0.7
LAMADR (n=1)	4.2	0.2	0.1	3.6	17.2	29.3	0.9	3.4	3.1	0.4	0.7	0.7	0.5	0.1	2.6	1.5	5.8	14.7	0.3	0.0	2.1	5.1	0.0	2.7	0.5	0.3
LAMQJH (n=1)	4.8	0.3	0.1	3.0	17.7	28.1	0.7	4.6	2.3	0.4	0.7	0.6	0.4	0.1	2.2	1.5	4.7	14.4	0.4	0.0	2.2	6.8	0.0	3.5	0.5	0.2
LAMQUI (n=1)	0.2	0.1	0.1	3.8	24.4	25.8	1.2	2.4	1.9	0.4	0.7	0.8	1.1	0.3	2.2	1.6	4.0	14.9	0.2	0.0	2.2	7.3	0.0	3.1	0.6	0.8
LAMRUR (n=1)	4.9	0.2	0.3	4.2	20.3	32.0	1.1	3.2	2.7	0.3	0.8	0.6	0.0	0.0	3.0	1.7	4.0	11.3	0.3	0.0	1.8	3.8	0.0	2.7	0.6	0.3
LANPSF (n=1)	4.7	0.3	0.0	3.9	20.8	30.7	0.8	4.1	2.0	0.3	0.8	0.6	0.5	0.1	2.4	1.4	4.5	11.4	0.3	0.0	2.1	4.7	0.0	2.6	0.6	0.4
LANBCH (n=1)	4.6	0.2	0.1	3.9	21.3	30.3	1.1	4.0	2.0	0.3	0.8	0.6	0.4	0.1	2.5	1.8	3.3	12.4	0.3	0.0	1.8	4.8	0.0	2.5	0.7	0.3

Table 51: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF:

deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	D5	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LALHCV (n=1)	59.1 ± 3.3	9 ± 0	8.9 ± 0.4	9 ± 0	7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.5 ± 1.4	4 ± 0.2	129.2 ± 3.5				
LANBCH (n=30)	51.9 ± 3.1	9 ± 0.2	8.5 ± 0.7	9 ± 0.2	8.1 ± 0.7	3 ± 0	3 ± 0	5 ± 0	8 ± 0.7	5 ± 0.8	127.7 ± 6.6			35.2 ± 2.8	30 ± 2
LANPMZ (n=74)	57.3 ± 1.6	9 ± 0	8.9 ± 0.4	9 ± 0	7.3 ± 0.7	3 ± 0	3 ± 0	5 ± 0	7.9 ± 1.1	4 ± 0	125.3 ± 5				
LAOIKC (n=29)	65.2 ± 2.1	9 ± 0	7.1 ± 1.7	7.4 ± 1.1	6.2 ± 2.9	3 ± 0	3 ± 0	5 ± 0	7.7 ± 2	4.1 ± 0.4	118.8 ± 5.8	4.9 ± 0.5	5.3 ± 2.3		
LALHBO (n=29)	61.3 ± 1.9	9 ± 0	7.9 ± 1.3	8.8 ± 0.4	7.3 ± 0.5	3 ± 0	3 ± 0	5.1 ± 0.4	8 ± 0.9	3.9 ± 0.4	128.3 ± 3.3				
LALRCH (n=30)	61.3 ± 1.8	9 ± 0	8.2 ± 1.4	7.8 ± 1	7.7 ± 1.9	3 ± 0	3 ± 0	5 ± 0	8.2 ± 1.3	3.8 ± 0.6	125 ± 3	5.2 ± 0.4	3.7 ± 1		
LALWPA (n=29)	60.4 ± 3.9	9 ± 0	9 ± 0	9 ± 0	7.6 ± 1.1	3 ± 0	3 ± 0	5 ± 0	7.6 ± 1.1	4.9 ± 0.8	127.3 ± 3.7				
LALXOL (n=15)	60.7 ± 4.6	9 ± 0	8.9 ± 0.7	8.2 ± 0.8	7.6 ± 0.9	3 ± 0	3 ± 0	5 ± 0	8.4 ± 0.9	4 ± 0.6	126 ± 4.6	5.4 ± 0.4	3.3 ± 1.3		
LALXVM (n=30)	56.7 ± 4	8.7 ± 1.3	9 ± 0	8.9 ± 0.4	7.3 ± 1.3	3 ± 0	3 ± 0	5 ± 0	7.3 ± 1.4	5.6 ± 1.3	121 ± 24.4				
LALZGS (n=24)	56.9 ± 6	9 ± 0	8.4 ± 1.3	9 ± 0	7.8 ± 1	3 ± 0	3 ± 0	5 ± 0	7.9 ± 0.9	5.1 ± 0.7	128.4 ± 5.5				
LAMADR (n=44)	59.9 ± 2.6	9 ± 0	8.7 ± 0.9	8.8 ± 0.5	7.5 ± 1.1	3 ± 0	3 ± 0	5 ± 0	8.2 ± 1.2	4 ± 0.2	126.5 ± 5.3				
LAMQJH (n=30)	60.3 ± 3.1	9 ± 0	8.5 ± 1.1	8.3 ± 0.6	8.1 ± 0.9	3 ± 0	3 ± 0	5 ± 0	8.6 ± 0.9	3.9 ± 0.3	124 ± 4.6	5.3 ± 0.4	4.7 ± 1.3		
LAMQUI (n=36)	59.6 ± 1.9	9 ± 0	9 ± 0	9 ± 0	8.3 ± 0.7	3 ± 0	3 ± 0	5 ± 0	8.3 ± 0.7	4.9 ± 0.5	129.3 ± 4.5				
LAMRUR (n=30)	55.1 ± 2.9	9 ± 0	9 ± 0	9 ± 0	7.3 ± 0.8	3 ± 0	3 ± 0	5 ± 0	7.4 ± 0.9	5.7 ± 0.8	126.8 ± 4.4				
LANPSF (n=30)	59.1 ± 3.3	9 ± 0	8.9 ± 0.4	9 ± 0	7 ± 0.9	3 ± 0	3 ± 0	5 ± 0	7.5 ± 1.4	4 ± 0.2	129.2 ± 3.5				
WT Kumily (n=46)														34.9 ± 1.1	32.2 ± 1

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Fatty acid profiles of T2 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

Field data from the T3 seed indicate that field values are lower for EPA and DHA than what was observed in the greenhouse, with values ranging from six to thirteen percent of the total fatty acid content of the seed for EPA and DHA combined. These data show a difference in seed oil content observed in field studies compared to the greenhouse (e.g. comparing Table 54 with Table 51), see also Example 10. Results of this analysis are described in Example 20.

Table 52: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field in field trials, corresponding to USDA zones 3a-4b and zone 5a , of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANBCH (n=30)	5.1 ± 0.2	0.4 ± 0	0 ± 0	2.6 ± 0.1	26 ± 1.9	31.3 ± 1	0.7 ± 0.1	6.2 ± 0.4	1.2 ± 0.2	0.2 ± 0	0.7 ± 0	0.7 ± 0	0.7 ± 0.1	0.2 ± 0	1.9 ± 0.1	1.1 ± 0.1	3.4 ± 0.3	8.9 ± 0.8	0.3 ± 0	0 ± 0	1.4 ± 0.2	4.7 ± 0.4	0 ± 0	1.5 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
LANPMZ (n=30)	4.9 ± 0.1	0.3 ± 0	0 ± 0	2.4 ± 0.1	30.7 ± 1.5	32.1 ± 0.8	0.3 ± 0	6.9 ± 0.3	0.5 ± 0.1	0.1 ± 0	0.7 ± 0	0.9 ± 0	1.4 ± 0.3	0.5 ± 0.1	2 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	5.8 ± 0.5	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.3 ± 0.2	0 ± 0	1 ± 0.1	0.4 ± 0.1	0.5 ± 0.1
LAOIKC (n=31)	5.2 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.1	26 ± 2.2	31.4 ± 1.1	0.8 ± 0.1	5.5 ± 0.3	1.4 ± 0.2	0.2 ± 0	0.7 ± 0	0.7 ± 0	0.6 ± 0.1	0.1 ± 0	2.4 ± 0.2	1.1 ± 0.1	5.7 ± 0.5	8 ± 0.6	0.2 ± 0	0 ± 0	1.1 ± 0.1	4.1 ± 0.3	0 ± 0	1.2 ± 0.2	0.1 ± 0.1	0.4 ± 0.1
LALHBO (n=29)	5.1 ± 0.2	0.2 ± 0.1	0 ± 0	2.5 ± 0.2	28 ± 6.4	30.3 ± 2	0.6 ± 0.2	6.7 ± 1.1	1.1 ± 0.4	0.2 ± 0.1	0.7 ± 0	0.8 ± 0.1	0.9 ± 0.2	0.3 ± 0.1	1.9 ± 0.4	1.1 ± 0.2	3.8 ± 1	8.5 ± 2.1	0.3 ± 0	0 ± 0	0.9 ± 0.3	4.2 ± 1	0 ± 0	1.2 ± 0.3	0.1 ± 0.1	0.4 ± 0.1
LALWPA (n=30)	5 ± 0.4	0.3 ± 0	0 ± 0	2.2 ± 0.2	21.3 ± 2.7	28.8 ± 1.8	0.8 ± 0.1	5.5 ± 0.5	1.6 ± 0.3	0.3 ± 0.1	0.7 ± 0	0.8 ± 0	1 ± 0.2	0.3 ± 0	2.3 ± 0.3	1.3 ± 0.1	6 ± 1.4	11.1 ± 1.3	0.3 ± 0	0 ± 0	1.5 ± 0.1	5.9 ± 0.7	0 ± 0	2 ± 0.3	0.3 ± 0.2	0.5 ± 0.1
LALXOL (n=29)	5.1 ± 0.2	0.3 ± 0.1	0 ± 0	2.6 ± 0.2	26.8 ± 1.9	29.7 ± 1.8	0.4 ± 0.1	6 ± 0.8	0.8 ± 0.2	0.2 ± 0.1	0.7 ± 0	1 ± 0.1	2.1 ± 0.7	0.5 ± 0.2	1.9 ± 0.2	1 ± 0.1	5.5 ± 1.6	8.5 ± 1	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.8 ± 0.6	0 ± 0	1.1 ± 0.2	0 ± 0	0.8 ± 0.2
LALXVM (n=30)	5 ± 0.3	0.3 ± 0	0 ± 0	2.4 ± 0.1	29 ± 4	29.9 ± 1.3	0.5 ± 0.1	6.3 ± 0.9	0.8 ± 0.2	0.2 ± 0	0.7 ± 0	0.9 ± 0.1	1.3 ± 0.4	0.4 ± 0.1	2 ± 0.3	1.1 ± 0.1	4.4 ± 1.3	7.7 ± 1.8	0.3 ± 0	0 ± 0	0.8 ± 0.2	3.9 ± 0.9	0 ± 0	1.1 ± 0.3	0.2 ± 0.1	0.6 ± 0.2
LALZGS (n=29)	5.1 ± 0.2	0.2 ± 0.1	0 ± 0	2.6 ± 0.2	25.1 ± 1.4	30.6 ± 1.2	0.6 ± 0.1	5.9 ± 0.4	1 ± 0.1	0.2 ± 0	0.7 ± 0	0.8 ± 0	1 ± 0.2	0.3 ± 0.1	2.3 ± 0.1	1.2 ± 0.1	5.7 ± 0.5	8.8 ± 0.5	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	4.5 ± 0.2	0 ± 0	1.2 ± 0.1	0 ± 0.1	0.7 ± 0.1
LAMADR (n=30)	5.1 ± 0.1	0.2 ± 0.1	0 ± 0	2.7 ± 0.2	25.9 ± 1	32 ± 1.2	0.7 ± 0.1	5.9 ± 0.4	1.3 ± 0.2	0.2 ± 0	0.7 ± 0	0.8 ± 0	0.7 ± 0.1	0.2 ± 0	2.1 ± 0.1	1 ± 0.1	4.7 ± 0.6	8 ± 0.7	0.3 ± 0	0 ± 0	1.3 ± 0.1	4.1 ± 0.3	0 ± 0	1.2 ± 0.1	0.2 ± 0.1	0.4 ± 0.1
LAMQUI (n=30)	4.7 ± 0.2	0.3 ± 0.1	0 ± 0	2.5 ± 0.2	28.8 ± 1.9	29 ± 1.6	0.8 ± 0.2	5.7 ± 0.5	1.2 ± 0.2	0.2 ± 0	0.6 ± 0	0.9 ± 0.1	1 ± 0.3	0.2 ± 0.1	1.7 ± 0.3	0.8 ± 0.1	4.6 ± 0.9	7.6 ± 1	0.2 ± 0	0 ± 0	2.1 ± 0.3	4.6 ± 0.7	0 ± 0	1.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.1
LAMRUR (n=29)	5.1 ± 0.3	0.3 ± 0	0 ± 0	2.6 ± 0.1	28.5 ± 2.3	32 ± 1.9	0.8 ± 0.2	5.8 ± 0.8	1.3 ± 0.2	0.2 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.4 ± 0.1	0 ± 0.1	2.1 ± 0.2	1 ± 0.1	3.8 ± 0.6	7.2 ± 0.7	0.2 ± 0.1	0 ± 0	1.3 ± 0.2	3.9 ± 0.4	0 ± 0	1.2 ± 0.2	0.3 ± 0.1	0.3 ± 0.1
LANPSF (n=28)	5.1 ± 0.2	0.3 ± 0.1	0 ± 0	2.6 ± 0.1	25.9 ± 1.8	31.4 ± 1.4	0.7 ± 0.2	6.1 ± 0.7	1.2 ± 0.3	0.2 ± 0	0.7 ± 0	0.8 ± 0	0.7 ± 0.2	0.2 ± 0.1	1.9 ± 0.2	1 ± 0.1	3.9 ± 0.9	7.6 ± 1.1	0.3 ± 0	0 ± 0	1.7 ± 0.2	4.2 ± 0.4	0 ± 0	1.4 ± 0.2	0.6 ± 0.2	0.4 ± 0.1
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ± 4.2	23.3 ± 1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

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Table 53: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in field trials, corresponding to USDA zones 3a-4b and zone 5a , of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANBCH (n=30)	5.3	0.4	0.1	2.5	25.5	29.5	0.7	6.1	1.3	0.3	0.6	0.7	0.8	0.2	1.9	1.1	3.7	10.1	0.2	0.0	1.4	5.2	0.0	1.6	0.2	0.4
LANPMZ (n=30)	5.1	0.3	0.0	2.3	27.1	31.5	0.2	6.9	0.4	0.1	0.7	0.9	2.1	0.8	2.4	1.5	3.0	6.8	0.3	0.0	0.9	4.1	0.0	1.4	0.5	0.6
LAOIKC (n=31)	5.3	0.3	0.0	2.6	23.3	32.0	0.8	5.3	1.5	0.2	0.7	0.7	0.5	0.1	2.5	1.2	6.1	8.9	0.2	0.0	1.2	4.5	0.0	1.4	0.1	0.4
LALHBO (n=29)	5.2	0.4	0.1	2.5	25.8	29.0	0.8	5.5	1.5	0.3	0.7	0.7	0.7	0.2	2.1	1.2	4.5	10.6	0.3	0.0	1.1	4.9	0.0	1.6	0.0	0.4
LALWPA (n=30)	5.9	0.4	0.0	2.0	15.4	27.9	0.8	5.2	2.5	0.5	0.7	0.7	0.9	0.2	2.7	1.4	7.1	13.2	0.4	0.0	1.8	6.9	0.0	2.7	0.0	0.4
LALXOL (n=29)	5.2	0.2	0.0	2.7	24.3	28.8	0.5	5.9	0.9	0.2	0.7	1.0	2.3	0.6	1.9	1.1	5.3	10.1	0.3	0.0	0.9	4.8	0.0	1.4	0.0	1.0
LALXVM (n=30)	4.9	0.3	0.1	2.4	26.7	28.2	0.5	5.3	0.8	0.2	0.7	0.9	1.6	0.5	2.1	1.2	5.3	10.2	0.3	0.0	0.8	4.5	0.0	1.4	0.1	0.9
LALZGS (n=29)	5.4	0.3	0.0	2.4	24.5	29.1	0.6	5.6	1.1	0.2	0.6	0.8	1.1	0.3	2.5	1.3	6.3	9.6	0.2	0.0	1.0	4.9	0.0	1.5	0.0	0.7
LAMADR (n=30)	5.2	0.3	0.0	2.6	25.6	30.1	0.7	5.5	1.4	0.3	0.7	0.8	0.8	0.1	2.1	1.0	5.4	9.1	0.3	0.0	1.3	4.5	0.0	1.5	0.0	0.4
LAMQUI (n=30)	4.4	0.4	0.0	2.3	26.7	26.2	0.9	5.3	1.2	0.2	0.6	0.9	1.3	0.3	2.0	1.0	5.0	9.8	0.2	0.0	2.4	6.0	0.0	1.7	0.6	0.7
LAMRUR (n=29)	6.3	0.3	0.0	2.8	34.5	23.3	0.5	4.5	1.1	0.2	0.5	0.5	0.4	0.1	2.3	1.2	3.5	9.6	0.2	0.0	1.1	5.1	0.0	1.7	0.0	0.3
LANPSF (n=28)	5.0	0.3	0.1	2.5	26.6	29.7	0.8	4.9	1.6	0.3	0.7	0.7	0.5	0.1	1.9	1.0	5.4	9.2	0.3	0.0	1.7	4.6	0.0	1.5	0.4	0.3

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Table 54: Phenotypic rating of T2 plants cultivated in the field of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LANBCH (n=30)	37.9 ± 1.2	27.9 ± 0.9
LANPMZ (n=30)	38.7 ± 1	27.8 ± 0.9
LAOIKC (n=31)	38.8 ± 1.1	28.1 ± 2.7
LALHBO (n=29)	37.9 ± 1.4	28.2 ± 0.7
LALWPA (n=30)	36.5 ± 1.4	28 ± 0.7
LALXOL (n=29)	38.4 ± 1.1	27.7 ± 0.6
LALXVM (n=30)	38.3 ± 1.1	27.7 ± 1
LALZGS (n=29)	39.5 ± 0.7	27.2 ± 0.6
LAMADR (n=30)	38.7 ± 0.9	27.6 ± 0.5
LAMQUI (n=30)	38.3 ± 0.9	28.7 ± 0.8
LAMRUR (n=29)	38.3 ± 1.1	27.8 ± 0.8
LANPSF (n=28)	38.4 ± 1.1	27.6 ± 0.8
WT Kumily (n=60)	38.7 ± 1.1	

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Fatty acid profiles copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in greenhouses during winter

The data indicate that EPA and DHA are still being synthesized by the plant in the T4 seed/generation.

Table 55: Copy number measurement of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T3 plants that were measured per event. As the T3 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth.

Event	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-i-Atss18_c-d6Elo(Pp_GA2) near the right T-DNA border.							Copy number assays targeting the T-DNA of VC-LLM337-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target j-t-PvARC-p3 located near the left T-DNA border and target c-d4Des(Eg_GA) near the right T-DNA border.							
	c-AHAS	j-t-E9-p3-2	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-t-CaMV_p-LuCnl 2	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)	j-t-PvARC-p3	c-d4Des(Tc_GA)	c-o3Des(Pi_GA2_SNP)	j-i-Atss15_c-o3Des(Pi_GA2)	c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Eg_GA)
LANPMZ (n=74)	1.9					2	2.02		1.94				2.04		1.88

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Table 56: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in greenhouses of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=30)	5.3 ± 0.3	0.2 ± 0	0 ± 0	3.6 ± 0.8	25.2 ± 5.3	38.1 ± 2.8	0.5 ± 0.1	4.1 ± 0.6	1.1 ± 0.3	0.1 ± 0.1	0.8 ± 0.2	0.7 ± 0	0.5 ± 0.1	0.1 ± 0.1	1.9 ± 0.2	0.9 ± 0.1	3.8 ± 0.6	6.9 ± 1.3	0.3 ± 0.1	0 ± 0	1.2 ± 0.4	2.6 ± 0.5	0 ± 0	1.5 ± 0.6	0.2 ± 0.1	0.3 ± 0.1

Table 57: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in greenhouses of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	4.9	0.2	0.0	4.0	19.5	38.5	0.5	3.6	1.6	0.2	1.0	0.7	0.4	0.0	1.6	0.8	4.8	9.2	0.4	0.0	1.7	3.8	0.0	2.5	0.0	0.2

Table 58: Phenotypic rating of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LANPMZ (n=30)	42.4 ± 2	8.2 ± 0.8	8.7 ± 0.7	9 ± 0	8 ± 0.7	3 ± 0	5 ± 0	5 ± 0		5.2 ± 0.8	128.5 ± 8.5				

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Fatty acid profiles and phenotypes of T3 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 8a-9a during winter

The data indicate that in the field the T4 seed are making EPA and DHA, but at lower levels than seen in the summer field trial (Part D). The greenhouse data show higher oil content compared to the summer field trials (Comparison of Table 61 with Table 54). This data was analyzed in detail in Example 20.

Table 59: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in the field in USDA growth zones 8a-9a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=56)	5.2 ± 0.5	0.3 ± 0.1	0.1 ± 0.2	2.6 ± 0.5	35.6 ± 2.7	30.4 ± 1.8	0.3 ± 0.1	7.1 ± 0.5	0.5 ± 0.3	0.1 ± 0.1	0.6 ± 0	0.9 ± 0.1	1.3 ± 0.3	0.4 ± 0.1	1.6 ± 0.2	1 ± 0.1	2.5 ± 0.3	4.5 ± 0.6	0.2 ± 0.1	0 ± 0	0.5 ± 0.3	2.6 ± 0.3	0 ± 0	0.7 ± 0.2	0.3 ± 0.1	0.5 ± 0.1
LAOIKC (n=16)	5.3 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.2	32.6 ± 2.2	29.9 ± 0.9	0.7 ± 0.1	5.9 ± 0.4	1 ± 0.2	0.2 ± 0	0.6 ± 0	0.7 ± 0	0.6 ± 0.1	0 ± 0	2.1 ± 0.1	1 ± 0.1	4.6 ± 0.4	6.2 ± 0.5	0.2 ± 0	0 ± 0	0.8 ± 0.1	3.3 ± 0.2	0 ± 0	0.9 ± 0.2	0 ± 0.1	0.4 ± 0.1
WT Kumily (n=83)	5.1 ± 0.5	0.4 ± 0.1	0.1 ± 0.1	2.1 ± 0.5	59.1 ± 1.8	21.3 ± 1.5	0 ± 0.1	9.5 ± 0.7	0 ± 0.1	0 ± 0	0.6 ± 0.1	1 ± 0.1	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.3 ± 0	0 ± 0	0 ± 0.1	0 ± 0.1	0 ± 0	0 ± 0	5.1 ± 0.5	0.4 ± 0.1

Table 60: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the field in USDA growth zones 8a-9a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	4.9	0.3	0.0	2.3	31.2	32.0	0.2	7.6	0.4	0.1	0.6	0.9	1.6	0.6	1.6	1.1	2.8	5.7	0.2	0.0	0.8	3.3	0.0	1.1	0.4	0.5
LAOIKC (n=1)	5.4	0.3	0.0	2.4	29.6	30.3	0.7	5.5	1.4	0.2	0.5	0.7	0.5	0.0	2.2	1.1	5.4	7.3	0.2	0.0	1.0	3.6	0.0	1.3	0.0	0.3

Table 61: Phenotypic rating of T3 plants cultivated in the field in USDA growth zones 8a-9b of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content in T4 seeds harvested from T3 plants (% of seed weight), Protein: Protein content in T4 seeds harvested from T3 plants (% of seed cake without oil)

Event	Oil	Protein
LANPMZ (n=56)	43.9 ± 7.8	23.2 ± 3.2
LAOIKC (n=16)	42.6 ± 4	23.1 ± 3

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WT Kumily (n=83)	45.3 ± 3.9	

Fatty acid profiles and phenotypes of T4 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during summer.

The data indicate that through the T5 generation the transformants are still producing EPA and DHA at a level consistent with the field trial in summer 2012 (described in part D). An additional observation is that the oil levels are comparable between these two field trials.

Table 62: Fatty acid profiles of T5 seeds harvested from T4 plants cultivated in the filed in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=124)	4.5 ± 0.4	0.2 ± 0.1	0 ± 0	2.8 ± 0.4	34.7 ± 3.9	33 ± 4.8	0.1 ± 0.2	6.5 ± 0.8	0.6 ± 0.2	0.1 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	0.7 ± 0.3	0.2 ± 0.1	1.7 ± 0.3	1.2 ± 1.3	2.8 ± 0.6	4.3 ± 1.9	0.3 ± 0.1	0 ± 0	0.7 ± 0.4	2.8 ± 0.6	0 ± 0.2	0.8 ± 0.3	0.1 ± 0.2	0.4 ± 0.2
WT Kumily (n=56)	4.5 ± 0.5	0.1 ± 0.1	0 ± 0	1.8 ± 1	60 ± 3.8	22.5 ± 3.6	0.6 ± 1.5	7.9 ± 2.2	0.1 ± 0.1	0 ± 0	0.8 ± 0.2	1 ± 0.4	0.1 ± 0	0 ± 0	0.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0.1	0.2 ± 0.2	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 63: Fatty acid profiles of one T5 seed batch per event harvested from T3 plants cultivated in the field in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T5 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LANPMZ (n=1)	5.6	0.3	0.0	2.7	21.8	32.3	0.0	6.3	0.9	0.0	0.8	0.8	1.5	0.5	2.2	1.5	4.7	8.0	0.4	0.0	1.6	5.1	0.0	2.7	0.0	0.4

Table 64: Phenotypic rating of T4 plants cultivated in the field in USDA growth zones 3a-4b and 5a of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM337-1qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content in T4 seeds harvested from T3 plants (% of seed weight), Protein: Protein content in T4 seeds harvested from T3 plants (% of seed cake without oil)

Event	Oil	Protein
LANPMZ (n=124)	39.7 ± 2.7	25.8 ± 1.4
WT Kumily (n=56)	40.6 ± 2.3	26.4 ± 1.3

Example 12: Plants containing the T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM338-3qcz rc (combination C in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNA. To this end, the two different plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc containing two different T-DNAs where cloned into Agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2197-1qcz or VC-LLM338-3qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2197-1qcz. Only those plants where kept, that also contained the T-DNA of plasmid VC-LLM338-3qcz rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2197-1qcz as well as the T-DNA of plasmid VC-LLM338-3qcz rc combine all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2197-1qcz and the function of each element are listed in Table 1. The genetic elements of VC-LLM338-3qcz rc and the function of each element was listed in Table 5. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2197-1qcz and VC-LLM338-3qcz rc that are required for EPA and DHA synthesis are additionally listed in Table 65.

Table 65: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2197-1qcz	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d15Des(Ch_ERTp_GA)	VC-LLM338-3qcz rc	1238	Delta-15 desaturase from *Cochliobolus heterostrophus* C5
c-d6Des(Ot_febit)	VC-LJB2197-1qcz	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	VC-LJB2197-1qcz	872	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	VC-LJB2197-1qcz	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2197-1qcz	1319	Delta-5 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
			Thraustochytrium sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LLM338-3qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-d5Elo(Ot_GA3)	VC-LLM338-3qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM338-3qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)	VC-LLM338-3qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc cultivated in greenhouses during summer

The data in Table 67 and Table 68 indicate that for this construct the increase in EPA and DHA, when comparing single copy to double copy events, was more subtle, but double copy events still had an increase in EPA and DHA over single copy events. As observed in the other examples, there was no significant observed alteration of the phenotype of the plants bearing the T-DNA from both constructs..

Table 66: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Category of T0 plants	Copy number assays targeting the T-DNA of VC-LJB2197-1qcz. Assays are listed according to the position of the assay target along the T-DNA, with assay of target c-AHAS was located near the left T-DNA border and assay of target c-d6Elo(Pp_GA) near the right T-DNA border.		Copy number assays targeting the T-DNA of VC-LLM338-3qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with assay of target c-d4Des(Eg_GA) was located near the left T-DNA border and assay of target c-d15Des(Ch_ERTp_GA) near the right T-DNA border.	
	c-AHAS	c-d6Elo(Pp_GA)	c-d4Des(Eg_GA)	c-d15Des(Ch_ERTp_GA)
sc (n=240)	1.0	1.0	1.2	1.2
dc (n=117)	1.6	1.7	1.8	2.0

Table 67: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=240)	5.8 ± 0.9	0.4 ± 0.3	0 ± 0	2.8 ± 0.9	34.2 ± 5.7	30.5 ± 3.3	0.2 ± 1.2	7.9 ± 1.6	0.7 ± 0.5	0.2 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	1 ± 0.6	0.3 ± 0.3	1.6 ± 0.6	1 ± 0.5	3.5 ± 1.7	3 ± 1.1	0.4 ± 0.2	0 ± 0	0.5 ± 0.3	2.1 ± 0.8	0 ± 0	1.4 ± 0.6	0.4 ± 0.4	
dc (n=117)	5.8 ± 0.9	0.4 ± 0.2	0 ± 0	2.9 ± 0.4	31.7 ± 6.5	30.3 ± 3.3	0.2 ± 0.2	7.9 ± 1.9	1.3 ± 1.1	0.4 ± 0.5	0.9 ± 0.1	0.8 ± 0.1	0.7 ± 0.5	0.2 ± 0.2	1.8 ± 1	1.1 ± 0.6	4 ± 2.1	3.2 ± 1.3	0.4 ± 0.2	0 ± 0	0.8 ± 0.4	2.6 ± 0.9	0 ± 0.1	1.8 ± 0.6	0.6 ± 0.6	

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Table 68: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	6.0	0.3	0.1	3.8	19.5	34.3	0.2	4.4	1.0	0.2	0.8	0.7	1.9	0.6	2.0	1.3	8.0	8.9	0.3	0.0	0.7	3.1	0.0	1.9	0.0	
dc (n=1)	5.8	0.4	0.0	3.2	19.5	29.5	0.1	8.5	1.6	0.7	0.9	0.7	1.7	0.7	1.6	1.2	6.5	7.2	0.4	0.0	0.9	4.9	0.0	3.9	0.3	

Table 69: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2197-1qcz and VC-LLM338-3qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 66. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=240)	81.4 ± 12.3	1.3 ± 0.6	1.9 ± 1.1	2.2 ± 1.3	2.1 ± 1.3	3 ± 0.1	4.6 ± 0.7	5 ± 0.3	3.1 ± 1.4	3.6 ± 0.9	93.7 ± 15.3	2.3 ± 0.7	7.4 ± 1.7
dc (n=117)	78.6 ± 10.1	1.3 ± 0.5	1.9 ± 1	2.3 ± 1.4	2.4 ± 1.2	3 ± 0.1	4.6 ± 0.7	5 ± 0	3.1 ± 1.4	3.9 ± 4.6	93.1 ± 17.4	2.2 ± 0.6	7.5 ± 1.5

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Example 13: Plants containing the T-DNAs of plasmid VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc (combination D in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc containing two different T-DNAs where cloned into agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2755-2qcz rc or VC-LLM391-2qcz rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2755-2qcz rc. Only those plants where kept, that also contained the T-DNA of plasmid VC-LLM391-2qcz rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2755-2qcz rc as well as the T-DNA of plasmid VC-LLM391-2qcz rc combine all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2755-2qcz rc and the function of each element are listed in Table 2. The genetic elements of VC-LLM391-2qcz rc and the function of each element was listed in Table 6. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc that are required for EPA and DHA synthesis are additionally listed Table 70.

Table 70: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2755-2qcz rc	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d5Des(Tc_GA2)	VC-LJB2755-2qcz rc	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-d6Des(Ot_febit)	VC-LJB2755-2qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Tp_GA2)	VC-LJB2755-2qcz rc	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-o3Des(Pi_GA2)	VC-LJB2755-2qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LJB2755-2qcz rc	1091	Omega-3 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
			Pythium irregulare
c-d5Elo(Ot_GA3)	VC-LLM391-2qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Eg_GA)	VC-LLM391-2qcz rc	1625	Delta-4 desaturase from *Euglena gracilis*
c-d4Des(Tc_GA)_T564G	VC-LLM391-2qcz rc	1559	Delta-4 desaturase from *Thraustochytrium* spp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during summer

The data in Table 72, Table 73 and Table 74 show that this combination of constructs was able to insert the T-DNA into the genome, but EPA and DHA accumulation was again at a more subtle level than observed in previous examples. None the less the constructs successfully recapitulated the pathway to generate EPA and DHA and ARA with no impact on the aerial portion of the plant.

Table 71: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering that each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
sc (n=184)	1.0							1.1	1.0	1.0		1.0		1.1	
dc (n=6)	1.0							2.1	1.1	1.5				2.2	

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Table 72: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering that each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=184)	5.4 ± 0.5	0.3 ± 0.1	0.2 ± 0.1	3 ± 0.4	39.5 ± 5.1	31.2 ± 2.9	0.4 ± 0.1	6.1 ± 0.9	1.3 ± 0.5	0.3 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0 ± 0	1.3 ± 0.4	0.8 ± 0.2	1.3 ± 0.6	4.2 ± 1.4	0.3 ± 0	0 ± 0	0.4 ± 0.2	1.5 ± 0.5	0 ± 0	0.6 ± 0.3	0.2 ± 0.1	
dc (n=6)	4.7 ± 0.8	0.3 ± 0.1	0.1 ± 0.1	2.9 ± 0.4	34.2 ± 5.2	30.6 ± 3.9	0.8 ± 0.6	5.8 ± 0.9	3.8 ± 3.4	0.9 ± 0.8	0.7 ± 0.1	0.8 ± 0	0.1 ± 0.1	0 ± 0	1.9 ± 1.3	1.2 ± 0.8	1.2 ± 1	5.9 ± 5.8	0.3 ± 0	0 ± 0	0.5 ± 0.2	1.9 ± 1.3	0 ± 0	0.6 ± 0.4	0.6 ± 0.6	

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Table 73: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	3.8	0.1	0.1	2.8	41.9	22.2	1.0	5.5	1.9	0.4	0.8	1.0	0.1	0.0	2.1	1.6	1.4	8.4	0.4	0.0	0.5	2.6	0.0	1.0	0.3	
dc (n=1)	3.6	0.1	0.0	2.6	25.9	28.4	1.3	4.7	3.9	1.0	0.7	0.8	0.1	0.1	2.3	1.6	2.7	14.0	0.4	0.0	0.6	3.6	0.0	1.4	0.2	

Table 74: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 71. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=184)	61.7 ± 7.8	1 ± 0	1.4 ± 1.2	1.6 ± 1.4	2.9 ± 2.5	3 ± 0	3 ± 0.1	5 ± 0	3.2 ± 2.5	3.7 ± 0.7	121.7 ± 9.4	4.9 ± 0.6	5.5 ± 2.1
dc (n=6)	63.7 ± 8	1 ± 0	1 ± 0	1.8 ± 1.3	2.8 ± 0.4	3 ± 0	3 ± 0	5 ± 0	2.8 ± 1	3.3 ± 1	123.3 ± 5.2	4.9 ± 1.2	5.5 ± 1.8

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during winter.

The data on Table 75, Table 76, Table 77, Table 78, Table 79, Table 80, Table 81 and Table 82 demonstrate that this pair of constructs was successful in recapitulating the pathway to generate VLC-PUFA (C20 and C22, including EPA, DHA and ARA). The copy number for each gene varied from homozygous single insertion of the T-DNA to insertions of parts of the T-DNA's and/or deletions of the T-DNA after insertion into the genome. The fatty acid profile indicated that some events (see Table 78, event LAPCSC) were able to accumulate up to 18 percent EPA and DHA combined). Table 75 indicates that LAPCSC was largely homozygous for a single insertion of each T-DNA with the exception of region of j-p-LuPXR_i-Atss15 on construct VC-LJB2755-2qcz, which contained at least four copies of the regions around that marker. The data presented on Table 81 indicate there was no obvious alteration of the phenotype of the plants bearing T-DNA corresponding to the constructs VC-LJB2755-2qcz and VC-LLM391-2qcz rc.

Table 75: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings using half-kernel analysis, where the correlation of VCL-PUFA levels with copy number was employed to select for homozygous plants, or on case of multilocus events to selecect for plants where one or more loci are homozygous. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.								Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAPCTC (n=11)	1.3 (T0: 1.1)			2.3		2.1		4.6 (T0: 3.6)	1.7 (T0: 1)	N/A (T0: 1.2)		1.7		1.7 (T0: 1.1)	
LAPCSC (n=11)	1.4 (T0: 1)			2.7		2.6		5.1 (T0: 4)	1.6 (T0: 1)	N/A (T0: 1)		1.7		1.6 (T0: 1.1)	
LAPYTJ (n=15)	1.7 (T0: 0.9)			3.2		3.3		3.2 (T0: 2.1)	1.7 (T0: 1.1)	N/A (T0: 1.1)		1.8		1.7 (T0: 1.1)	
LAQKQ5 (n=15)	1.5 (T0: 1.1)			1.7		1.4		2 (T0: 1.3)	1.4 (T0: 1)	N/A (T0: 1.1)		1.5		1.4 (T0: 1)	
LAPARV (n=15)	2 (T0: 1.1)			1.9		2.0		2 (T0: 1.1)	1.2 (T0: 1.1)	N/A (T0: 0.9)		1.2		1.2 (T0: 1.1)	
LAPCMY (n=15)	2 (T0: 1)			2.0		2.1		2.2 (T0: 1.1)	1.7 (T0: 1)	N/A (T0: 1.1)		1.7		1.9 (T0: 1.2)	
LAPBOW (n=15)	2 (T0: 1)			1.9		2.1		2 (T0: 1)	2 (T0: 1)	N/A (T0: 1.2)		1.9		2.1 (T0: 1.1)	
LAPAWA (n=15)	1.9 (T0: 1.1)			2.0		2.0		2.1 (T0: 1.1)	1.5 (T0: 1)	N/A (T0: 1)		1.5		1.5 (T0: 1.2)	
LAPBYW (n=13)	2 (T0: 1)			2.0		2.2		2.3 (T0: 1)	1.3 (T0: 1)	N/A (T0: 0.8)		1.3		1.5 (T0: 1.1)	
LAPQEP (n=15)	1.4 (T0: 0.9)			2.8		2.6		4 (T0: 3.1)	1.3 (T0: 0.9)	N/A (T0: 1.2)		1.4		1.4 (T0: 1.1)	
LAODDN (n=15)	2.1 (T0: 1.1)			2.0		2.1		2.1 (T0: 1.1)	2 (T0: 1)	N/A (T0: 1.1)		2.0		2.1 (T0: 1.1)	
LAPAUX (n=10)	1.5 (T0: 1)			1.6		1.7		1.7 (T0: 1)	1 (T0: 0.9)	N/A (T0: 0.8)		1.0		1.1 (T0: 0.6)	
LAPZOJ (n=10)	1.8 (T0: 1)			2.1		1.9		2 (T0: 1.1)	1.8 (T0: 1)	N/A (T0: 1.2)		1.9		1.9 (T0: 1)	

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Table 76: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, dc: all T1 plants where the average of all copy number assays listed in this table was 3.51-4.49, tc: all T1 plants where the average of all copy number assays listed in this table was 5.51-6.49. The number of T1 plants fullfilling these criteria are displayed in parentheses.

Category of T1 plants	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)
sc (n=140)	1.8			2.1		2.1		2.6	1.6			1.6		1.6

Table 77: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPCTC (n=11)	5.1 ± 0.2	0.3 ± 0	0 ± 0	3 ± 0.3	28.2 ± 5.5	28.9 ± 1.6	1.3 ± 0.2	5 ± 0.2	3 ± 0.5	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.1 ± 0	2.2 ± 0.4	1.8 ± 0.5	1.2 ± 0.4	10.9 ± 2.1	0.4 ± 0	0 ± 0	0.6 ± 0.2	3.5 ± 1.1	0 ± 0	1.3 ± 0.4	0.4 ± 0.2	0.2 ± 0.1
LAPCSC (n=11)	5.1 ± 0.1	0.3 ± 0	0 ± 0	3.2 ± 0.4	27.9 ± 4.2	28.8 ± 2.3	1.3 ± 0.2	4.9 ± 0.4	3.2 ± 0.6	0.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.1 ± 0	2.1 ± 0.4	1.7 ± 0.5	1.3 ± 0.3	11.4 ± 2.4	0.4 ± 0.1	0 ± 0	0.6 ± 0.1	3.4 ± 1	0 ± 0	1.2 ± 0.3	0.3 ± 0.1	0.2 ± 0.1

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LAPYTJ (n=15)	5.9 ± 0.3	0.3 ± 0.1	0 ± 0	3.3 ± 0.6	26.5 ± 1.9	34 ± 1.6	0.9 ± 0.2	4.9 ± 0.7	2.1 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0	0 ± 0	0 ± 0	2.2 ± 0.3	1.3 ± 0.3	2.9 ± 0.5	8.4 ± 1.7	0.1 ± 0.1	0 ± 0	0.9 ± 0.3	2.8 ± 0.5	0 ± 0	1.2 ± 0.3	0.2 ± 0.2	0.1 ± 0.1
LAQKQ5 (n=15)	5.3 ± 0.3	0.3 ± 0.1	0 ± 0	3.2 ± 0.6	32.7 ± 8	29.7 ± 2.7	1.1 ± 0.3	5.3 ± 0.5	2.6 ± 0.6	0.6 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	1.7 ± 0.6	1.3 ± 0.5	1.1 ± 0.2	8.8 ± 3.2	0.2 ± 0.2	0 ± 0	0.5 ± 0.3	2.4 ± 0.6	0 ± 0	1 ± 0.4	0.2 ± 0.2	0.1 ± 0.1
LAPARV (n=15)	5.3 ± 0.2	0.3 ± 0	0 ± 0	3.5 ± 0.3	26.5 ± 1.9	36 ± 1.2	0.7 ± 0.1	5.4 ± 0.3	1.9 ± 0.2	0.4 ± 0.1	0.9 ± 0.1	0.7 ± 0	0 ± 0	0 ± 0	1.9 ± 0.3	1.4 ± 0.2	1.7 ± 0.3	8.4 ± 1.6	0.4 ± 0	0 ± 0	0.6 ± 0.2	2.4 ± 0.5	0 ± 0	1.2 ± 0.3	0.3 ± 0.2	
LAPCMY (n=15)	6.2 ± 1.3	0.3 ± 0.1	0 ± 0	2.4 ± 1.4	34 ± 8.6	26.7 ± 14.5	0.5 ± 0.3	6.5 ± 1.4	1.9 ± 0.4	0.4 ± 0.1	1 ± 0.2	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	2.5 ± 0.5	1.8 ± 0.4	1.6 ± 0.4	7.9 ± 2	0.4 ± 0.1	0 ± 0	0.6 ± 0.3	2.6 ± 0.8	0 ± 0	1.2 ± 0.4	0.3 ± 0.2	
LAPBOW (n=15)	5.1 ± 0.6	0.2 ± 0.1	0 ± 0	2.9 ± 0.2	27.3 ± 2.6	34.9 ± 0.9	0.7 ± 0	5.6 ± 0.6	1.9 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0 ± 0	2 ± 0.3	1.4 ± 0.2	1.6 ± 0.1	7.8 ± 1.1	0.3 ± 0	0 ± 0	0.9 ± 0.2	3.1 ± 0.7	0 ± 0	1.3 ± 0.4	0.6 ± 0.1	0.1 ± 0
LAPAWA (n=15)	5.2 ± 0.1	0.3 ± 0	0 ± 0	3.1 ± 0.2	28.3 ± 1.3	35.9 ± 1.1	0.7 ± 0.1	5.3 ± 0.3	1.7 ± 0.2	0.3 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	2.1 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	7.4 ± 0.7	0.3 ± 0	0 ± 0	0.7 ± 0.2	2.2 ± 0.5	0 ± 0	1 ± 0.3	0.5 ± 0.2	
LAPBYW (n=13)	5.6 ± 0.3	0.3 ± 0.1	0 ± 0	2.6 ± 1.2	28.4 ± 1.2	36 ± 1.2	0.7 ± 0.1	5.4 ± 0.3	1.7 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0.1 ± 0.1	1.9 ± 0.2	1.4 ± 0.2	1.6 ± 0.1	7.9 ± 0.8	0.4 ± 0.1	0 ± 0	0.5 ± 0.2	2 ± 0.4	0 ± 0	1 ± 0.2	0.3 ± 0.2	
LAPQEP (n=15)	5 ± 0.3	0.3 ± 0	0 ± 0	2.7 ± 0.3	38.1 ± 5.8	26.7 ± 4	1.1 ± 0.3	6.1 ± 0.9	1.2 ± 0.2	0.3 ± 0	0.8 ± 0.1	0.8 ± 0.1	0.2 ± 0.1	0.1 ± 0	1.6 ± 0.4	1.5 ± 0.4	0.7 ± 0.2	7.7 ± 1.6	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.8 ± 0.7	0 ± 0	0.7 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
LAODDN (n=15)	5.2 ± 0.6	0.2 ± 0.1	0 ± 0	3.1 ± 0.3	28 ± 2	35.8 ± 1.1	0.7 ± 0.1	5.9 ± 0.4	1.6 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0 ± 0	0 ± 0	2 ± 0.2	1.4 ± 0.2	1.2 ± 0.1	7.1 ± 0.9	0.4 ± 0	0 ± 0	0.8 ± 0.1	2.8 ± 0.4	0 ± 0	1.1 ± 0.3	0.6 ± 0.1	0.1 ± 0
LAPAUX (n=10)	5.5 ± 0.5	0.3 ± 0	0 ± 0	2.8 ± 0.3	31.3 ± 7.3	34.9 ± 2.5	0.5 ± 0.1	6.1 ± 0.6	1.5 ± 0.5	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0	0 ± 0	1.7 ± 0.5	1.2 ± 0.4	1.7 ± 0.5	8.3 ± 3.4	0.4 ± 0	0 ± 0	0.2 ± 0.1	1.1 ± 0.4	0 ± 0	0.4 ± 0.2	0 ± 0.1	
LAPZOJ (n=10)	5.6 ± 0.6	0.3 ± 0	0 ± 0	3 ± 0.3	34.1 ± 6.8	33.5 ± 2.8	0.6 ± 0.2	6.5 ± 0.6	1.3 ± 0.3	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0 ± 0	0 ± 0	1.6 ± 0.4	1.1 ± 0.3	1 ± 0.2	5.5 ± 1.5	0.2 ± 0.1	0 ± 0	0.4 ± 0.2	2.2 ± 0.6	0 ± 0	0.8 ± 0.3	0.3 ± 0.2	0 ± 0

Table 78: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPCTC (n=1)	5.1	0.3	0.0	2.7	25.2	29.7	1.3	4.8	2.6	0.5	0.8	0.6	0.3	0.1	2.4	1.9	1.9	14.1	0.3	0.0	0.4	3.3	0.0	1.0	0.2	0.3
LAPCSC (n=1)	5.0	0.3	0.0	3.4	20.8	25.8	1.7	5.2	3.5	1.0	0.8	0.6	0.3	0.1	2.5	2.7	1.0	16.1	0.3	0.0	0.6	5.3	0.0	1.9	0.4	0.3
LAPYTJ (n=1)	5.5	0.3	0.0	3.0	24.0	31.1	1.1	4.9	2.4	0.5	0.8	0.6	0.0	0.0	2.4	1.7	2.8	10.8	0.3	0.0	1.1	4.1	0.0	1.7	0.5	0.3
LAQKQS (n=1)	5.8	0.3	0.0	3.6	24.4	31.3	1.4	4.7	3.9	0.9	0.8	0.6	0.0	0.0	2.2	1.8	1.5	13.8	0.0	0.0	0.0	1.8	0.0	1.0	0.0	0.0

LAPARV (n=1)	5.3	0.2	0.0	3.2	24.3	34.2	0.6	5.6	2.0	0.5	0.8	0.6	0.1	0.0	2.0	1.5	2.3	11.9	0.3	0.0	0.6	2.4	0.0	1.1	0.3	
LAPCMY (n=1)	8.1	0.5	0.0	0.0	51.4	1.0	0.0	9.5	2.3	0.6	1.2	1.0	0.1	0.0	2.9	2.1	2.4	12.5	0.5	0.0	0.3	2.6	0.0	0.8	0.0	
LAPBOW (n=1)	3.8	0.1	0.0	2.3	21.5	33.7	0.7	6.7	2.3	0.6	0.7	0.7	0.1	0.1	2.2	1.8	2.0	10.8	0.4	0.0	1.5	5.2	0.0	2.1	0.7	0.1
LAPAWA (n=1)	5.3	0.3	0.0	3.4	26.2	36.5	0.7	4.8	1.8	0.4	0.9	0.7	0.0	0.0	2.7	1.9	1.7	8.7	0.4	0.0	0.5	1.7	0.0	0.9	0.3	
LAPBYW (n=1)	5.5	0.3	0.0	0.0	29.4	36.1	0.6	5.8	1.8	0.4	0.8	0.8	0.1	0.0	1.9	1.5	1.7	9.5	0.4	0.0	0.4	1.7	0.0	0.8	0.3	
LAPQEP (n=1)	4.9	0.4	0.0	2.3	36.6	23.9	1.5	5.7	1.3	0.4	0.7	0.8	0.3	0.1	1.9	1.6	1.0	10.9	0.4	0.0	0.3	3.7	0.0	0.7	0.3	0.3
LAODDN (n=1)	3.8	0.1	0.0	2.6	24.1	36.0	0.8	5.7	2.0	0.4	0.8	0.7	0.0	0.0	2.3	1.8	1.6	9.4	0.4	0.0	1.1	3.8	0.0	1.6	0.6	0.1
LAPAUX (n=1)	5.5	0.3	0.0	3.1	25.6	33.6	0.7	5.4	1.9	0.4	0.8	0.6	0.1	0.0	2.4	1.7	2.3	12.8	0.3	0.0	0.2	1.5	0.0	0.5	0.0	
LAPZOJ (n=1)	5.7	0.3	0.0	2.1	25.8	31.3	0.4	7.9	2.2	0.7	0.6	0.6	0.0	0.0	1.3	1.0	2.1	9.3	0.0	0.0	1.0	5.6	0.0	2.2	0.0	0.0

Table 79: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=143)	5.4 ± 0.7	0.3 ± 0.1	0 ± 0	2.9 ± 0.7	29.6 ± 5.5	32.8 ± 6.2	0.8 ± 0.3	5.6 ± 0.8	1.9 ± 0.6	0.4 ± 0.2	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0 ± 0	2 ± 0.4	1.5 ± 0.3	1.5 ± 0.5	8.3 ± 2	0.3 ± 0.1	0 ± 0	0.6 ± 0.3	2.6 ± 0.7	0 ± 0	1.1 ± 0.4	0.4 ± 0.2	0.1 ± 0.1

Table 80: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.1	0.3	0.0	2.7	25.2	29.7	1.3	4.8	2.6	0.5	0.8	0.6	0.3	0.1	2.4	1.9	1.9	14.1	0.3	0.0	0.4	3.3	0.0	1.0	0.2	0.3

Table 81: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes(#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LAPCTC (n=11)	67.5 ± 1.5	1 ± 0	1 ± 0	1 ± 0	1.8 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	129.5 ± 3.5	3.6 ± 0.6	3.6 ± 0.9		
LAPCSC (n=11)	68.6 ± 3.3	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.6 ± 0.8	126.4 ± 3.2	3.2 ± 0.3	3.9 ± 0.5		
LAPYTJ (n=15)	72.3 ± 7.1	1 ± 0	1.4 ± 1.1	1.1 ± 0.5	1.6 ± 0.8	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.7	4 ± 0	123 ± 4.9	3.6 ± 0.3	3.5 ± 0.8		
LAQKQS (n=15)	67.4 ± 4.4	1 ± 0	1.4 ± 0.8	1.1 ± 0.3	3.1 ± 1.6	3 ± 0	3 ± 0	5 ± 0	1.8 ± 1.5	4 ± 0	130.3 ± 4.8	3.8 ± 0.5	3.7 ± 1		
LAPARV (n=15)	68.1 ± 2.6	1 ± 0	1.2 ± 0.8	1 ± 0	2.3 ± 2.1	3 ± 0	3 ± 0	5 ± 0	1.3 ± 0.9	4 ± 0	128 ± 3.2	4 ± 0.6	3.9 ± 1.3		
LAPCMY (n=15)	68.7 ± 2.4	1 ± 0	1 ± 0	1 ± 0	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	3.9 ± 0.5	130.7 ± 1.8	3.6 ± 0.4	3.3 ± 0.7		
LAPBOW (n=15)	68.1 ± 0.9	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	127.3 ± 3.2	4 ± 0.4	3.7 ± 1	35.3 ± 1.3	29.2 ± 0.3
LAPAWA (n=15)	66.5 ± 1.4	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	128.7 ± 3.5	3.7 ± 0.4	2.6 ± 0.5		
LAPBYW (n=13)	65.7 ± 2.6	1 ± 0	1.2 ± 0.8	1.2 ± 0.6	1.7 ± 0.6	3 ± 0	3 ± 0	5 ± 0	1.2 ± 0.6	3.8 ± 0.6	128.1 ± 5.2	4 ± 0.5	3.9 ± 1.7		
LAPQEP (n=15)	66.9 ± 2.6	1 ± 0	1.2 ± 0.8	1 ± 0	1.7 ± 0.7	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	128 ± 4.6	3.2 ± 0.3	3.3 ± 0.6		
LAODDN (n=15)	69.3 ± 2	1 ± 0	1 ± 0	1 ± 0	1.2 ± 0.4	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	125.7 ± 3.2	3.9 ± 0.5	3.8 ± 1.2	36.6 ± 1.2	27.9 ± 0.4
LAPAUX (n=10)	70 ± 4.5	1 ± 0	1.3 ± 0.9	1 ± 0	3.1 ± 2.3	3 ± 0	3 ± 0	5 ± 0	2.4 ± 2.1	3.8 ± 0.6	130 ± 2.4	4.3 ± 0.5	4.5 ± 1.4		
LAPZOJ (n=10)	68 ± 6.7	1 ± 0	1.2 ± 0.6	1 ± 0	1.1 ± 0.3	3 ± 0	3 ± 0	5 ± 0	1 ± 0	4 ± 0	126.5 ± 3.4	3.8 ± 0.6	3.3 ± 0.9		

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Table 82: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 76. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=143; n=30 for oil and protein)	67.9 ± 3	1 ± 0	1.1 ± 0.5	1 ± 0.2	1.7 ± 1.1	3 ± 0	3 ± 0	5 ± 0	1.1 ± 0.7	3.9 ± 0.3	127.9 ± 4	3.6 ± 0.4	3.5 ± 1.1	36 ± 1.4	28.5 ± 0.8

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Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during summer.

The data in Table 83 indicate the copy number of the selected events was a single insertion which was homozygous in the T3 seed. Fatty acid profile measurements, see Table 84 and Table 85, indicated the combination of T-DNAs from VC-LJB2755-2qcz and VC-LLM391-2qcz rc are capable of bringing in the VLC-PUFA pathway to successfully accumulate ARA, EPA and DHA. The data on Table 86 show that there was no significant impact on the aerial portion of the plant caused by VC-LJB2755-2qcz and VC-LLM391-2qcz rc.

Table 83: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. As the T2 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-	j-i-Atss14_c-	c-d6Elo(Tp_GA)	j-i-Atss1_c-	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAPBOW (n=54)	1.9	2.0					2.0	2.4	2.0				2.0		2.0
LAODDN (n=63)	1.9	2.0					2.0	2.1	2.0				2.0		1.9

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Table 84: Fatty acid profiles of T3 seeds harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T3 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=54)	5.3 ± 0.3	0.3 ± 0.1	0.2 ± 0.1	3.4 ± 0.2	26.8 ± 1.9	35.6 ± 2.1	0.6 ± 0.1	6.1 ± 0.8	1.6 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.8 ± 0.3	1.4 ± 0.3	1.4 ± 0.2	7.1 ± 0.8	0.3 ± 0.1	0 ± 0	1 ± 0.1	3.2 ± 0.5	0 ± 0	1.3 ± 0.3	0.6 ± 0.2	0.1 ± 0.1
LAODDN (n=63)	5.4 ± 0.4	0.3 ± 0.1	0.2 ± 0.1	3.5 ± 0.4	27.7 ± 2.4	35.4 ± 1.8	0.6 ± 0.1	6.5 ± 0.8	1.4 ± 0.2	0.4 ± 0	0.8 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.8 ± 0.2	1.4 ± 0.2	1 ± 0.2	6.8 ± 0.6	0.3 ± 0.1	0 ± 0	0.8 ± 0.1	3 ± 0.3	0 ± 0	1.1 ± 0.3	0.6 ± 0.1	0.1 ± 0.1
WT Kumily (n=46)	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0 ± 0	0 ± 0	0.9 ± 0	1.2 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0.5 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 85: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.7	0.3	0.2	3.3	24.1	34.3	0.6	6.1	2.0	0.5	0.7	0.6	0.0	0.0	2.1	1.7	1.6	8.7	0.1	0.0	1.1	3.8	0.0	1.7	0.7	0.0
LAODDN (n=1)	5.3	0.3	0.2	3.1	25.1	34.7	0.7	6.0	1.8	0.5	0.7	0.7	0.0	0.0	2.2	2.0	1.1	8.7	0.3	0.0	0.8	3.6	0.0	1.5	0.7	0.1

Table 86: Phenotypic rating of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=54)	34.8 ± 2.9	30.8 ± 2.3
LAODDN (n=63)	36.6 ± 3	28.6 ± 2.4

WT Kumily (n=46)	34.9 ± 1.1	32.2 ± 1

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during the summer

Field data for the T3 seed from the events carrying the T-DNA from VC-LJB2755-2qcz and VC-LLM391-2qcz rc, shown in Table 87 and Table 88, indicate that the plants are capable of making VLC-PUFAs in the field (ARA, EPA and DHA), though not at the level observed in the greenhouse. However, there was also a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 89 with Table 86). These observations are in agreement with previous examples where it was observed that increased oil contents in the field grown plants concomitant with a decrease in VLC-PUFAs, in particular EPA, DHA and ARA. A more detailed description of the observations regarding oil content and VLC-PUFAs is given in Example 20.

Table 87: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field, corresponding to USDA growth zones 3a-4b and 5a, for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=31)	5.3 ± 0.2	0.4 ± 0.1	0 ± 0	2.4 ± 0.1	31.9 ± 1.6	33.3 ± 1	0.6 ± 0.1	7.7 ± 0.5	1.4 ± 0.2	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.5 ± 0.1	0.9 ± 0.1	1.6 ± 0.2	5.5 ± 0.5	0.2 ± 0.1	0 ± 0	0.9 ± 0.1	3.1 ± 0.3	0 ± 0	0.8 ± 0.1	0.4 ± 0.1	0.1 ± 0.1
LAODDN (n=31)	5.1 ± 0.2	0.4 ± 0	0.1 ± 0	2.5 ± 0.2	32.1 ± 2.3	33.4 ± 1.4	0.6 ± 0.1	7.9 ± 0.5	1.3 ± 0.2	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.2 ± 0.1	0.1 ± 0.1	1.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	5.7 ± 0.7	0.3 ± 0	0 ± 0	0.8 ± 0.1	3.1 ± 0.4	0 ± 0	0.8 ± 0.1	0.4 ± 0.1	0.1 ± 0
WT Kumily (n=60)	5 ± 0.3	0.4 ± 0	0.1 ± 0.1	2 ± 0.1	56.2 ± 4.2	23.3 ± 1.7	0 ± 0.1	9.5 ± 0.7	0.1 ± 0.2	0 ± 0	0.7 ± 0	1.1 ± 0.1	0.1 ± 0.1	0 ± 0	0.2 ± 0.3	0.1 ± 0.3	0.2 ± 0.5	0.4 ± 1.1	0.3 ± 0.1	0 ± 0	0 ± 0.2	0.2 ± 0.6	0 ± 0	0 ± 0.2		

Table 88: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.5	0.4	0.1	2.3	30.3	32.9	0.6	7.6	1.8	0.5	0.6	0.7	0.1	0.0	1.6	1.0	1.7	6.5	0.1	0.0	1.0	3.5	0.0	1.1	0.2	0.1
LAODDN (n=1)	5.4	0.4	0.0	2.6	30.1	33.3	0.7	6.9	1.5	0.4	0.6	0.6	0.1	0.0	1.5	0.9	1.8	6.8	0.2	0.0	1.0	3.5	0.0	0.9	0.4	0.1

Table 89: Phenotypic rating of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=31)	38.7 ± 1.2	28 ± 1.1
LAODDN (n=31)	38.3 ± 1.4	27.9 ± 1.2

WT Kumily (n=60)	38.7 ± 1.1	

Fatty acid profiles, copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in greenhouses during winter.

T4 seed from T3 plants from the event LAODDN, which was homozygous for T-DNA from both VC-LJB2755-2qcz and VC-LLM391-2qcz rc (see Table 90) accumulated VLC-PUFAs (in particular ARA, EPA and DHA, see Table 91 and Table 92). The combination of EPA and DHA was up to approximately ten percent of the total fatty acid content in the seed for this event.

Table 90: Copy number measurement of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. As the T3 plants underwent two cylces of selecting homozygous plants, all plants of all events are homozygous for all T-DNA insertions. A copy number of ~2 therefore was indicative for one homozygous locus, a copy number of ~4 indicative for two homozygous loci or indicative for one homozygous locus containing two copies of the target gene measured by the assay, and so forth. Odd results of 3 and 5 indicate that at least some of the selected T1 plants carry a heterozygous locus.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LLM391-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.						
	c-AHAS	c-o3Des(Pl_GA)	j-i-Atss18_c-o3Des(Pl_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Eg_GA)	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
LAODDN (n=30)	1.92		2.0					1.9	1.8			1.9	2.1		1.9

Table 91: Fatty acid profiles of T4 seeds harvested from T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=30)	5.3 ± 0.5	0.3 ± 0	0 ± 0	3.3 ± 0.7	27.2 ± 2.5	37.7 ± 4.1	2.3 ± 5.1	5.1 ± 0.7	1.7 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0 ± 0	1.2 ± 0.2	0.3 ± 0.5	1.3 ± 0.2	6.5 ± 0.8	0.2 ± 0.2	0 ± 0	1 ± 0.3	2.7 ± 0.4	0 ± 0	1.3 ± 0.4	0.4 ± 0.1	0 ± 0

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Table 92: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LAODDN (n=1)	5.2	0.2	0.0	3.7	24.2	39.5	0.4	4.8	2.0	0.5	0.9	0.6	0.1	0.1	1.0	0.0	1.5	7.7	0.4	0.0	1.3	3.3	0.0	1.9	0.3	0.1

Table 93: Phenotypic rating of T3 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (9=deformed, 1=normal), DL: deformed leaf (9=deformed, 1=normal), DP: deformed plant (9=deformed, 1=normal), DS: deformed silique (9=deformed, 1=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH
LAODDN (n=30)	43.5 ± 4.1	8.5 ± 0.5	7.8 ± 0.8	9 ± 0	7.9 ± 1	3 ± 0	4.6 ± 0.7	4.5 ± 0.7	8.8 ± 0.4	5 ± 0.8	115 ± 12.5

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Fatty acid profiles, copy number measurements, and phenotypic observations of T3 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA growth zones 8a-9a in the winter.

Field data for T4 seed of two events carrying homozygous T-DNA insertions from VC-LJB2755-2qcz and VC-LLM391-2qcz rc (see Table 83 and Table 90 and Table 84, Table 87, Table 91) indicate these events do accumulate EPA, DHA and ARA when grown in the greenhouse and field, though as consistently observed, the field grown material did not accumulate the VLC-PUFAs (ARA, EPA, DHA) to the extent observed in the greenhouse (see Table 94 and Table 95 in comparison with Table 91, Table 92, Table 87 and Table 88). As observed in in Example 11 part F, higher oil content was observed compared to the summer field trials (Comparison Table 96 with Table 89). This phenomenon is analyzed in detail in Example 20.

Table 94: Fatty acid profiles of T4 seeds harvested from T3 cultivated in the field corresponding to USDA growth zones 8a-9a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=16)	5.3 ± 0.2	0.4 ± 0	0 ± 0	2.4 ± 0.2	36.1 ± 3.1	31.3 ± 2	0.5 ± 0.1	7.9 ± 0.5	1.3 ± 0.3	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0.1	0 ± 0	1.2 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	4.5 ± 0.5	0.2 ± 0	0 ± 0	0.7 ± 0.2	2.7 ± 0.2	0 ± 0	0.7 ± 0.2	0.3 ± 0.2	0.1 ± 0.1
LAODDN (n=47)	5.5 ± 0.6	0.5 ± 0.2	0.2 ± 0.3	2.7 ± 0.5	36.8 ± 3.2	30.3 ± 2.2	0.6 ± 0.2	7.9 ± 0.5	1.3 ± 0.3	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0	0.1 ± 0.1	0 ± 0	1 ± 0.1	0.8 ± 0.1	1.2 ± 0.2	4.7 ± 0.7	0.2 ± 0	0 ± 0	0.4 ± 0.3	2.6 ± 0.3	0 ± 0	0.6 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
WT Kumily (n=83)	5.1 ± 0.5	0.4 ± 0.1	0.1 ± 0.1	2.1 ± 0.5	59.1 ± 1.8	21.3 ± 1.5	0 ± 0.1	9.5 ± 0.7	0 ± 0.1	0 ± 0	0.6 ± 0.1	1 ± 0.1	0.1 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0.1	0.1 ± 0.1	0.3 ± 0	0 ± 0	0 ± 0.1	0 ± 0.1	0 ± 0	0 ± 0	5.1 ± 0.5	0.4 ± 0.1

Table 95: Fatty acid profiles of one T4 seed batch per event harvested from T3 plants cultivated in the field corresponding to USDA growth zones 8a-9a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T4 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAPBOW (n=1)	5.3	0.4	0.0	2.3	31.5	34.0	0.5	7.3	1.8	0.6	0.6	0.7	0.1	0.0	1.2	0.8	1.8	5.5	0.2	0.0	1.0	3.1	0.0	1.1	0.2	0.0
LAODDN (n=1)	5.6	0.4	0.0	2.1	33.8	31.1	0.7	8.0	1.5	0.5	0.5	0.7	0.1	0.0	1.2	0.8	1.6	6.2	0.1	0.0	0.7	3.3	0.0	1.0	0.2	0.1

Table 96: Phenotypic rating of T3 plants cultivated in the field corresponding to USDA growth zones 8a-9a for trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAPBOW (n=16)	42.9 ± 4.2	23.3 ± 3.1
LAODDN (n=47)	43.5 ± 3.8	22.7 ± 2.7

WT Kumily (n=83)	45.3 ± 3.9	

Fatty acid profiles, copy number measurements, and phenotypic observations of T4 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc cultivated in field trials in USDA zones 3a-4b and 5a during the summer.

The data indicate that through the T5 generation the event LAODDN was still producing EPA and DHA at a level consistent with the field trial (described in part D). Also oil content was comparable between these two field trials.

Table 97: Fatty acid profiles of T5 seeds harvested from T4 cultivated in the field corresponding to USDA growth zones 3a-3b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc The events are indicated in the first column, along with the number of T4 seed aliquots representing a plot where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=142)	4.7 ± 0.4	0.2 ± 0.1	0 ± 0	2.8 ± 0.4	34.8 ± 3.5	33.8 ± 4.8	0.3 ± 0.3	7.7 ± 1	1.1 ± 0.4	0.2 ± 0.2	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	1.2 ± 0.3	1.1 ± 1.2	1.4 ± 0.4	4.5 ± 1.9	0.3 ± 0.1	0 ± 0	0.5 ± 0.4	2.9 ± 0.5	0.1 ± 0.2	0.7 ± 0.2	0.1 ± 0.2	0.1 ± 0.1
WT Kumily (n=56)	4.5 ± 0.5	0.1 ± 0.1	0 ± 0	1.8 ± 1	60 ± 3.8	22.5 ± 3.6	0.6 ± 1.5	7.9 ± 2.2	0.1 ± 0.1	0 ± 0	0.8 ± 0.2	1 ± 0.4	0.1 ± 0	0 ± 0	0.2 ± 0.8	0 ± 0	0 ± 0	0 ± 0.1	0.2 ± 0.2	0 ± 0	0 ± 0.1	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0

Table 98: Fatty acid profiles of one T5 seed batch per event harvested from T4 plants cultivated in the field corresponding to USDA growth zones 3a-3b and 5a for field trials of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column. Fatty acid profiles of T5 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LAODDN (n=1)	4.5	0.2	0.0	2.6	30.3	30.8	0.4	5.8	0.6	0.1	0.6	0.9	0.3	0.4	2.4	1.3	3.7	8.1	0.3	0.0	0.0	4.2	1.2	1.2	0.0	0.0

Table 99: Phenotypic rating of T4 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LLM391-2qcz rc. The events are indicated in the first column, along with the number of field plots that were rated per event. Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	Oil	protein
LAODDN (n=47)	39.9 ± 4.4	25.4 ± 1.8
WT Kumily (n=56)	40.6 ± 2.3	26.4 ± 1.3

Example 14: Plants containing the T-DNAs of plasmid VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc (combination E in example 5) for production of EPA and DHA in seeds

In this example, the genetic elements required for EPA and DHA synthesis were transferred into the plant genome on two different T-DNAs. To this end, the two different plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc containing two different T-DNAs were cloned into Agrobacteria, and plant tissue was incubated according to example 5 at the same time with these two agrobacterial cultures that are identical apart from containing either VC-LJB2755-2qcz rc or VC-LTM217-1qcz rc rc. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LJB2755-2qcz rc. Only those plants were kept, that also contained the T-DNA of plasmid VC-LTM217-1qcz rc rc as confirmed by PCR, conducted as described in example 5. Only plants containing the T-DNA of plasmid VC-LJB2755-2qcz rc as well as the T-DNA of plasmid VC-LTM217-1qcz rc combined all the genetic elements required for EPA and DHA synthesis in seeds. The genetic elements of VC-LJB2755-2qcz rc and the function of each element are listed in Table 2. The genetic elements of VC-LTM217-1qcz rc and the function of each element were listed in Table 7. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LJB2755-2qcz rc and VC- LTM217-1qcz rc that were required for EPA and DHA synthesis are additionally listed Table 100.

Table 100: Combined list of genes essential of EPA and DHA synthesis carried by the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA)	VC-LJB2755-2qcz rc	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	VC-LJB2755-2qcz rc	1370	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Tp_GA2)	VC-LJB2755-2qcz rc	818	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	VC-LJB2755-2qcz rc	1319	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	VC-LJB2755-2qcz rc	1085	Omega-3-desaturase from *Phythophthora infestans*
c-o3Des(Pir_GA)	VC-LJB2755-2qcz rc	1091	Omega-3 desaturase from *Pythium irregulare*

Genes encoding enzmyes for EPA and DHA synthesis	Plasmid containing T-DNA with the gene	Length (bp)	Enzymatic function and source of encoded protein
c-d5Elo(Ot_GA3)	VC-LTM217-1qcz rc	902	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	VC-LTM217-1qcz rc	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA)	VC-LTM217-1qcz rc	1560	Delta-4 desaturase from *Thraustochytrium* spp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc cultivated in greenhouses during winter.

Table 102 and Table 103 indicate the single copy events for insertions of VC-LJB2755-2qcz and VC-LTM217-1qcz rc did not accumulate as much EPA and DHA as the double copy events. Table 103 indicates that the combined EPA and DHA content, for the highest producers in T1 seed, was in the range of 15 percent of the total fatty acid content of the seed (5% of the total seed fatty acid content being DHA and 10% being EPA).

Table 101: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LTM217-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.						
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Pl_GA)2	j-t-PvARC-p-LuCnl	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)
sc (n=139)	1.2	1.2					1.2	1.2	1.2		1.1	1.2			1.1
dc (n=77)	1.6	1.8			2.0		1.8	1.8	1.9		1.9	1.7			1.9

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Table 102: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 101. The number of T1 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=139)	5.1 ± 0.5	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.8	37.8 ± 7.2	32 ± 4.4	0.4 ± 0.3	4.6 ± 0.6	1.7 ± 1.2	0.3 ± 0.3	0.9 ± 0.1	0.8 ± 0.1	0 ± 0.1	0 ± 0	1.4 ± 0.6	0.8 ± 0.4	1.8 ± 0.7	4.8 ± 1.8	0.4 ± 0.1	0 ± 0.3	0.2 ± 0.2	0.8 ± 0.5	0.3 ± 0.4	1.4 ± 0.6	0.1 ± 0.2	0 ± 0.1
dc (n=77)	5 ± 0.4	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.6	35.5 ± 7.4	32.8 ± 4.4	0.5 ± 0.2	4.7 ± 0.8	1.8 ± 0.7	0.3 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0 ± 0.1	0 ± 0	1.5 ± 0.8	0.9 ± 0.5	1.7 ± 0.6	5.5 ± 2.6	0.3 ± 0.1	0 ± 0	0.3 ± 0.2	1 ± 0.5	0.4 ± 0.4	1.7 ± 0.6	0.2 ± 0.3	0 ± 0.1

Table 103: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 101. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.0	0.4	0.0	2.9	32.0	26.7	1.3	3.3	3.4	0.6	0.9	0.8	0.1	0.0	1.6	1.0	2.7	10.3	0.4	0.0	1.2	0.0	0.0	5.2	0.2	0.1
dc (n=1)	5.1	0.2	0.1	5.1	23.3	31.7	0.7	3.8	2.9	0.6	1.0	0.8	0.2	0.1	1.7	1.3	2.2	12.5	0.4	0.0	0.5	1.9	0.7	3.0	0.2	0.1

Table 104: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined inTable 101. The number of T1 plants fullfilling these criteria are displayed in parentheses. TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	TKW	SC	Oil	Protein
sc (n=139)	4.3 ± 0.5	5 ± 1.8	33.3 ± 1.6	30.5 ± 0.6

dc (n=77)	4.1 ± 0.5	5.3 ± 2	33.5 ± 3	29.7 ± 1.1

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc cultivated in greenhouses during summer.

Measurements carried out on plants from selected T1 events indicated single copy homozygous insertions of the T-DNA corresponding to VC-LJB2755-2qcz and VC-LTM217-1qcz rc (see table 104, in particular the table legend) and the T2 accumulates EPA and DHA in similar levels as the T1. Measurements on T2 seed (see Table 106 and Table 107) showed that EPA and DHA accumulated up to 13% of the total fatty acid content of the seed (with ca. 3% of the seed total fatty acid content being DHA).

Table 105: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Homozygous plants of the T1 generation of these two events have been selected using Half Kernel Analysis (Example 9). Based on the proven ability to discriminate homozygous and heterozygous seeds using half kernel analysis, it can be assumed all T1 plants in Table 106, Table 107, and Table 108 are homozygous, but no copy number analysis has been performed to confirm this.

Event	Copy number assays targeting the T-DNA of VC-LJB2755-2qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target j-p-LuPXR_i-Atss15 near the right T-DNA border.								Copy number assays targeting the T-DNA of VC-LTM217-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-d4Des(Eg_GA) located near the left T-DNA border and target j-i-Atss1_c-d5Elo(Ot_GA3) near the right T-DNA border.					
	c-AHAS	c-o3Des(Pi_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-i-Atss1_c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	j-p-LuPXR_i-Atss15	c-d4Des(Pl_GA)2	j-t-PvARC-p-LuCnI	j-i-Atss2_c-d4Des(Tc_GA3)	c-d4Des(Tc_GA)	j-p-PvARC5_t-BnFAE	c-d5Elo(Ot_GA3)
LBAIID (n=1)	1.1	1.0					1.0	1.0	1.2		1.0			1.1
LBAPPG (n=1)	1.0	1.0					1.0	1.0	1.2		1.1			0.9

Table 106: Fatty acid profiles of T2 seeds harvested from homozygous T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc The events are indicated in the first column, along with the number of T2 seed batches that where measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBAIID (n=20)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3.6 ± 0.3	23.6 ± 1.8	38.4 ± 0.9	0.5 ± 0.1	4.9 ± 0.3	2.1 ± 0.2	0.5 ± 0.1	0.9 ± 0.1	0.6 ± 0	0.1 ± 0	0.1 ± 0	1.2 ± 0.1	0.9 ± 0.1	1.8 ± 0.2	8.1 ± 0.8	0.4 ± 0	0 ± 0	0.7 ± 0.1	1.8 ± 0.2	0.8 ± 0.1	3.4 ± 0.4	0.3 ± 0.1	0 ± 0
LBAPPG (n=18)	5.6 ± 0.5	0.3 ± 0.1	0 ± 0	4.3 ± 0.3	24.2 ± 2.7	39.9 ± 1.2	0.3 ± 0.1	4.7 ± 0.3	2 ± 0.3	0.4 ± 0.1	1 ± 0.1	0.6 ± 0	0.1 ± 0	0 ± 0	1 ± 0.1	0.7 ± 0.2	1.8 ± 0.2	6.9 ± 1.2	0.4 ± 0	0 ± 0	0.6 ± 0.1	1.6 ± 0.2	0.6 ± 0.2	2.9 ± 0.5	0 ± 0.1	0 ± 0

Table 107: Fatty acid profiles of one T2 seed batch per event harvested from homozygous T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBAIID (n=1)	5.2	0.3	0.0	3.7	20.9	37.3	0.4	5.3	2.4	0.6	0.9	0.6	0.1	0.1	1.0	0.8	2.0	9.2	0.4	0.0	0.8	2.2	1.0	4.5	0.3	0.0
LBAPPG (n=1)	4.9	0.2	0.0	4.6	22.3	38.8	0.3	4.7	2.1	0.5	1.2	0.7	0.1	0.0	1.2	0.9	2.0	8.2	0.5	0.0	0.6	1.8	0.7	3.7	0.0	0.0

Table 108: Fatty acid profiles of T2 seeds harvested from homozygous T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LJB2755-2qcz rc and VC-LTM217-1qcz rc. Plants of all events combined have been grouped into the category "sc". The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n -7	16:3n -3	18:0	18:1n -9	18:2n -6	18:2n -9	18:3n -3	18:3n -6	18:4n -3	20:0	20:1n -9	20:2n -6	20:3n -3	20:3n -6	20:4n -3	20:4n -6	20:5n -3	22:0	22:1n -9	22:4n -6	22:5n -3	22:5n -6	22:6n -3	unknown peak a	unknown peak b
sc (n=38)	2.6 ± 0.2	0.1 ± 0	0 ± 0	2 ± 0.2	12 ± 1.1	19.5 ± 0.6	0.2 ± 0	2.4 ± 0.1	1 ± 0.1	0.2 ± 0	0.5 ± 0	0.3 ± 0	0 ± 0	0 ± 0	0.5 ± 0.1	0.4 ± 0.1	0.9 ± 0.1	3.7 ± 0.6	0.2 ± 0	0 ± 0	0.3 ± 0	0.8 ± 0.1	0.3 ± 0.1	1.6 ± 0.3	0.1 ± 0.1	0 ± 0

Example 15: Plants containing the T-DNA of plasmid RTP10690-1qcz_F for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid RTP10690-1qcz_F was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of RTP10690-1qcz_F. The genetic elements of RTP10690-1qcz_F and the function of each element are listed in Table 8. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of RTP10690-1qcz_F that required for EPA and DHA synthesis are additionally listed Table 109.

Table 109: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid RTP10690-1qcz_F.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNA of plasmids RTP10690-1qcz_F cultivated in greenhouses during winter

As Table 110 indicates there were fewer insertion events of this construct observed than for other constructs, with more single copy events than double copy, by approximately four fold. Fatty acid profile data on Table 111 and Table 112 indicated that DHA and EPA can accumulate up to 4% of the total seed fatty acid content in the T1 with similar performance between single and double copy events, within error. Table 113 demonstrates that there was no significant aerial phenotype associated with this construct in the T0.

Table 110: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of RTP10690-1qcz_F. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border.													
Category of T0 plants	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=52)	1.1	2.0	1.2	1.0	1.1	1.0		1.0	1.2	1.0	1.1	1.0	2.0	1.1
dc (n=12)	1.6	3.5	2.0	1.8	1.9	2.0		1.9	2.2	1.8	2.1	1.8	3.5	1.8

Table 111: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=52)	5.1 ± 0.7	0.3 ± 0.1	0 ± 0	3.1 ± 0.4	43.8 ± 7.3	28.3 ± 4.5	0.3 ± 0.2	5.1 ± 0.8	0.8 ± 0.4	0.1 ± 0.1	0.9 ± 0.1	1 ± 0.1	0.5 ± 0.6	0.2 ± 0.3	2.2 ± 0.7	1.1 ± 0.4	1.6 ± 0.5	3.2 ± 1	0.4 ± 0.1	0 ± 0	0.1 ± 0.1	1.1 ± 0.4	0 ± 0	0.5 ± 0.3	0.1 ± 0.1	0.2 ± 0.2
dc (n=12)	5.2 ± 0.4	0.3 ± 0.1	0 ± 0.1	3.5 ± 0.5	39 ± 6.7	30.1 ± 3	0.5 ± 0.2	4.5 ± 0.6	1.6 ± 0.6	0.2 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.3 ± 0.4	0.1 ± 0.1	3.1 ± 1	1.2 ± 0.4	2 ± 1.4	3.7 ± 1.3	0.4 ± 0	0 ± 0	0.3 ± 0.2	1.2 ± 0.4	0 ± 0	0.7 ± 0.3	0.2 ± 0.2	0.1 ± 0.2

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Table 112: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	5.5	0.3	0.0	4.1	27.2	33.6	0.7	4.1	1.9	0.3	0.9	0.7	0.3	0.2	4.5	2.1	2.7	5.8	0.4	0.0	0.3	2.2	0.1	1.4	0.4	0.2
dc (n=1)	5.3	0.3	0.0	3.8	31.9	32.9	0.6	3.8	1.6	0.2	0.9	0.8	0.4	0.2	3.6	1.5	2.7	5.7	0.4	0.0	0.3	1.7	0.0	1.1	0.2	0.3

Table 113: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 110. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=52)	61.4 ± 15.5	8.7 ± 1.2	8.7 ± 1.2	8.8 ± 0.9	7.6 ± 2.5	3 ± 0	5.4 ± 7.9	5.9 ± 6.9	7.3 ± 2.6	4.9 ± 1.3	123.8 ± 7.5			35.4 ± 2.4	30.1 ± 1.1
dc (n=12)	56.3 ± 9.6	9 ± 0	9 ± 0	9 ± 0	8.1 ± 2.1	3 ± 0	4.3 ± 1	5 ± 0	8.1 ± 2.1	5 ± 0.9	125.4 ± 5.4			34.8 ± 1.1	29.9 ± 1

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNA of plasmids RTP10690-1qcz_F cultivated in greenhouses during summer

Data in Table 115 and Table 116 indicate a similar performance of the T2 seed as that of the T1 seed with respect to EPA and DHA, see also Table 111 and Table 112 for comparison. The selected events all performed at a similar level and segregated for one to two copies of each gene.

Table 114: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

	Copy number assays targeting the T-DNA of RTP10690-1qcz_F. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.												
Event	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Ot febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
LBDGCA (n=94)	1.5 (T0: 1)	3.2 (T0: 2)	1.6 (T0: 1.4)	(T0: 1)	(T0: 1.2)	1.7 (T0: 0.9)	1.6	1.6 (T0: 1)	(T0: 1.2)	1.7 (T0: 1)	(T0: 1.1)	(T0: 1.1)	3.2 (T0: 2)	1.6 (T0: 1)
LBDGBP (n=20)	(T0: 1.1)	(T0: 2.1)	(T0: 1.4)	(T0: 1.2)	(T0: 1.3)	(T0: 1.1)		(T0: 1.1)	(T0: 1.3)	(T0: 1)	(T0: 1)	(T0: 1.2)	(T0: 2.1)	(T0: 1)
LBDIHN (n=114)	1.6 (T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.7)	(T0: 1.1)	(T0: 1.2)	1.7 (T0: 1)	1.6	1.6 (T0: 1.1)		1.7 (T0: 1)	(T0: 0.9)	(T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.1)

Table 115: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBDGCA (n=90)	5.7 ± 0.5	0.2 ± 0.1	0 ± 0.1	4.1 ± 0.7	36.4 ± 7.3	32.5 ± 3.9	0.5 ± 0.2	4.5 ± 0.9	1.7 ± 0.7	0.2 ± 0.1	1.1 ± 0.2	0.8 ± 0.1	0.2 ± 0.1	0 ± 0	2.6 ± 0.6	1 ± 0.2	1.9 ± 0.7	3.3 ± 1.2	0.4 ± 0.1	0 ± 0	0.4 ± 0.2	1.4 ± 0.5	0 ± 0	0.6 ± 0.3	0.3 ± 0.1	0.1 ± 0.1
LBDGBP (n=20)	5.4 ± 0.5	0.3 ± 0	0 ± 0	3.9 ± 0.4	25.4 ± 2.3	37.1 ± 1.6	0.5 ± 0	3.4 ± 0.2	2.4 ± 0.3	0.2 ± 0	1 ± 0.1	0.6 ± 0	0.1 ± 0	0 ± 0	2.3 ± 0.2	0.9 ± 0.2	3.8 ± 0.5	7.5 ± 1.6	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.4 ± 0.4	0 ± 0.2	1.7 ± 0.7	0 ± 0	0 ± 0
LBDIHN (n=110)	4.9 ± 0.4	0.2 ± 0.1	0 ± 0	3.6 ± 0.5	32.5 ± 7.6	34.5 ± 4.1	0.6 ± 0.2	4.4 ± 1	1.7 ± 0.7	0.2 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	2.3 ± 0.4	1.1 ± 0.4	2.7 ± 0.9	5.2 ± 1.6	0.4 ± 0.1	0 ± 0	0.4 ± 0.2	1.9 ± 0.6	0 ± 0	0.9 ± 0.4	0.2 ± 0.1	0.1 ± 0.1

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Table 116: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Events	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBDGCA (n=1)	5.5	0.2	0.0	3.3	29.2	31.8	0.9	5.3	2.8	0.4	0.7	0.7	0.1	0.1	2.9	1.2	3.6	5.9	0.3	0.0	0.6	2.6	0.0	1.3	0.4	0.1
LBDGBP (n=1)	5.2	0.3	0.0	3.9	20.3	35.1	0.5	3.6	2.7	0.3	1.0	0.6	0.1	0.0	2.6	1.3	4.4	10.8	0.4	0.0	0.7	3.3	0.0	3.0	0.0	0.0
LBDIHN (n=1)	5.0	0.3	0.0	3.5	23.8	36.3	0.6	2.6	2.6	0.2	0.9	0.6	0.1	0.0	2.8	1.1	4.1	9.1	0.4	0.0	1.0	2.8	0.0	2.1	0.0	0.1

Table 117: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid RTP10690-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event. Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Events	Oil	Protein
LBDGCA (n=90)	34.1 ± 3.1	30.1 ± 1.9
LBDIHN (n=110)	35.3 ± 2.7	29.9 ± 1.8

Example 16: Plants containing the T-DNA of plasmid RTP10691-2qcz for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid RTP10691-2qcz was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of RTP10691-2qcz. The genetic elements of RTP10691-2qcz and the function of each element are listed in Table 9. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of RTP10691-2qcz that required for EPA and DHA synthesis are additionally listed in Table 118.

Table 118: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid RTP10691-2qcz.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1196	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1337	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Thraustochytrium sp.

Example 17: Plants containing the T-DNA of plasmid VC-LTM595-1qcz rc for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid VC-LTM595-1qcz rc was cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LTM595-1qcz rc. The genetic elements of VC-LTM595-1qcz rc and the function of each element are listed in Table 10. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LTM595-1qcz rc that required for EPA and DHA synthesis are additionally listed Table 119.

Table 119: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid VC-LTM595-1qcz rc.

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Phythophthora infestans
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from *Thraustochytrium* sp.

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNA of plasmids VC-LTM595-1qcz rc cultivated in greenhouses during summer

Similar to VC-RTP10690-1qcz_F, the number of insertions of the entire T-DNA was not as high as obtained for the multi-construct transformations, see Table 120. Table 121 and Table 122 show the fatty acid profile measurements for single, double and triple copy T-DNA, and indicate that the double copy constructs perform marginally better than the single copy constructs and perhaps marginally better than the triple copy constructs in terms of EPA and DHA accumulation. The fatty acid profile data further indicate that the accumulation of EPA and DHA in the T1 seed was up to 10% of the total fatty acids with up to 2% of the total fatty acids in the seed being DHA. Phenotypic measurements, shown on Table 123, indicated some variability in flowering time (as represented by DFF).

Table 120: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T0 plants where the average of all copy number assays listed in this table except the assay c-d5Des(Tc_GA) was 0.51-1.49, dc: all T0 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T0 plants where the average of all copy number assays listed in this table was 2.51-3.49.

	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.													
Category of T0 plants	c-AHAS	j-p-BnSETL-v1_c-o3Des(Pir_GA)	c-d4Des(Pl_GA)2	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-PvARC5_t-BnFAE	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d5Des(Tc_GA)	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=24)	1.1		1.0	1.0	0.9	0.9	1.9	1.1		1.0	0.9	1.1	1.9	1.0
dc (n=7)	2.2		2.3	2.1	2.0	1.9	3.8	2.3		2.2	2.3	2.1	3.8	1.9
tc (n=3)	2.4		2.4	1.9	2.5	2.0	4.6	3.3		2.4	2.9	2.4	4.6	2.4

Table 121: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
sc (n=24)	5.2 ± 0.6	0.3 ± 0.1	0 ± 0	3 ± 0.4	41.2 ± 4.8	28.6 ± 2.3	0.3 ± 0.1	5.9 ± 0.6	0.6 ± 0.3	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	1.9 ± 0.7	1.2 ± 0.5	1.5 ± 0.3	3.5 ± 0.8	0.4 ± 0.1	0 ± 0	0.3 ± 0.1	1.2 ± 0.3	0 ± 0.1	1.1 ± 0.3	0.4 ± 0.2	0.3 ± 0.2
dc (n=7)	5.6 ± 1.4	0.3 ± 0.2	0 ± 0	3.7 ± 0.7	34.4 ± 7.3	31.2 ± 3.4	0.4 ± 0.2	5.7 ± 1.4	1.5 ± 0.5	0.2 ± 0.2	0.9 ± 0.2	0.8 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	2.7 ± 0.8	1.5 ± 1	1.6 ± 0.8	3.8 ± 2.2	0.4 ± 0	0 ± 0	0.6 ± 0.4	1.5 ± 0.8	0.2 ± 0.1	1.6 ± 0.6	0.7 ± 0.1	0.2 ± 0.1
tc (n=3)	4.1 ± 1.3	0.2 ± 0.1	0 ± 0	3 ± 0.1	45.4 ± 8.4	26.2 ± 2.2	0.4 ± 0.3	6 ± 0.9	1.7 ± 2	0.2 ± 0.3	0.8 ± 0	1 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	1.5 ± 0.4	0.7 ± 0.1	1.5 ± 0.7	3.3 ± 1.6	0.4 ± 0	0 ± 0	0.5 ± 0.3	1 ± 0.4	0.2 ± 0.1	1.1 ± 0.5	0.3 ± 0.1	0.2 ± 0.1

Table 122: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
sc (n=1)	6.3	0.4	0.0	3.4	34.6	29.8	0.4	5.3	1.0	0.1	0.8	0.8	0.6	0.3	2.9	1.6	1.8	4.6	0.3	0.0	0.5	1.6	0.2	1.6	0.7	0.3
dc (n=1)	4.9	0.2	0.0	2.8	25.8	33.9	0.3	4.8	1.7	0.3	0.7	0.7	0.5	0.2	3.2	1.7	2.8	7.4	0.4	0.0	1.2	2.9	0.2	2.3	0.8	0.3
tc (n=1)	4.5	0.2	0.0	3.1	37.8	28.0	0.7	5.7	3.9	0.5	0.8	0.9	0.1	0.0	1.6	0.6	1.8	4.8	0.4	0.0	0.8	1.3	0.3	1.5	0.4	0.1

Table 123: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. Considering each event in this generation was represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 120. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC
sc (n=24)	44.8 ± 17.3	9 ± 0	8.7 ± 1.1	9 ± 0	6.7 ± 2.4	3 ± 0	4.7 ± 0.8	5 ± 0	6.7 ± 2.4	4.7 ± 1	123.5 ± 7.7	4.4 ± 0.5	1.6 ± 0.9
dc (n=7)	47.1 ± 10.3	9 ± 0	8.7 ± 0.8	9 ± 0	4.7 ± 2.6	3 ± 0	5 ± 0	5 ± 0	4.7 ± 2.6	4.7 ± 1.1	122.9 ± 5.7	4.3 ± 0.9	2.1 ± 1.5
tc (n=3)	42.3 ± 2.5	9 ± 0	9 ± 0	9 ± 0	6 ± 4.4	3 ± 0	5 ± 0	5 ± 0	6 ± 4.4	5 ± 1.7	125 ± 10	4.2 ± 0.2	4.3 ± 3.2

Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNA of plasmids VC-LTM595-1qcz rc cultivated in greenhouses during winter

The data Table 124, Table 125 and Table 126 indicate that a variety of copy numbers for the genes contained on the T-DNA were obtained, with copy number values ranging from 1 to 3. The performance of the events selected for measurements of fatty acid profiles (see Table 127 and Table 128) was similar to that observed in the previous example with the EPA and DHA combined value being approximately ten percent of the total fatty acid content of the seed, for the upper end of values. The data in Table 129 indicate, as observed in prior generations for this construct (see example 17 part A), that there was some variation in flowering time among the various events.

Table 124: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings, using zygosity analysis as illustrated in Table 125, keeping only plants that are homozygous for the desired number of loci (which are indicated in the last column of Table 125). A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygosity was indicated if the average result of the selected T1 plants was about two fold higher than the the result oberved in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	Conclusion from individual assays: number of T-DNA copies inserted into the genome
LBEDTZ	4.1 (T0: 2.2)	4	4 (T0: 2)	4 (T0: 1.8)	(T0: 1.8)	4.3 (T0: 1.8)	8.1 (T0: 3.8)	3.8 (T0: 2.5)	5.7	5 (T0: 2.8)	4.7 (T0: 2.8)	5.5 (T0: 3.1)	8.1 (T0: 3.8)	4.1 (T0: 2)	partial double copy
LBEDUA	4.3 (T0: 2.7)	4.2	4 (T0: 2.3)	3 (T0: 2.2)	(T0: 2)	4.5 (T0: 2.3)	8 (T0: 4)	4 (T0: 2.4)	4.3	3.7 (T0: 2.2)	3.6 (T0: 2.4)	4 (T0: 2.1)	8 (T0: 4)	3.8 (T0: 2.4)	partial double copy
LBEDWU	4.2 (T0: 1.8)	3.4	3.3 (T0: 1.9)	2.7 (T0: 1.6)	(T0: 1.7)	3.6 (T0: 1.7)	6.7 (T0: 3.5)	3.2 (T0: 1.9)	3.2	3.3 (T0: 1.7)	3 (T0: 1.7)	3.3 (T0: 1.8)	6.7 (T0: 3.5)	3.9 (T0: 2.1)	partial double copy
LBEFME	(T0: 2.6)		(T0: 2.5)	(T0: 2.6)	(T0: 2.4)		(T0: 3.7)	(T0: 3)		(T0: 2.4)	(T0: 2.7)	(T0: 2)	(T0: 3.7)	(T0: 1.1)	partial double copy
LBEEIL	(T0: 2.4)		(T0: 2.7)	(T0: 2.5)	(T0: 2.5)		(T0: 3.7)	(T0: 2)		(T0: 1.9)	(T0: 2)	(T0: 1.8)	(T0: 3.7)	(T0: 2.1)	partial double copy
LBEEBO	(T0: 2.1)		(T0: 2.7)	(T0: 1.8)	(T0: 1.6)	(T0: 1.9)	(T0: 3.6)	(T0: 1.9)		(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 3.6)	(T0: 2.1)	partial double copy
LBERLW	(T0: 2.1)		(T0: 1.4)	(T0: 1.7)	(T0: 1.6)		(T0: 2.6)	(T0: 2)		(T0: 1.2)	(T0: 0.9)	(T0: 1.6)	(T0: 2.6)	(T0: 1.6)	partial double copy

Table 125: Observed Medelian segregation of the genotype of T1 seeds of events from construct VC-LTM595-1qcz rc. The segregation has been analysed at three positions of the T-DNA. For each position, the number of seedlings have been counted that have a copy number (arithmetically rounded) of 0, 1, 2, 3, 4, 5,

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6, 7, 8, 9, 10, 11 and 12. The of seedlings counted for each copy number category are separated by colon, displaying the categories in the following order: 0 : 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8 : 9 : 10 : 11 : 12. Listed are the observed copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. The observed frequencies for each assay have been compared against expected frequencies for various locus configurations listed in Table 136 using Chi-Square analysis. The last column displays the total number of loci that are segregating in the genome of a given event. Many events contain truncated insertions, as was evident when some assays indicate single copy insertion at e.g. the left border (e.g. event LBEDTZ), while other position on the T-DNA clearly indicate a double copy insertion that are either inserted in one locus (e.g. event LBEDTZ), or in two loci (e.g. event LBEDWU)

Event	Copy number ratios measured near the left border of the T-DNA using an assay targetting c-AHAS	Copy number ratios measured using T-DNA internal assays targeting reagions that had copy number results indicating truncated T-DNA insertions using assay A04 (target: j-i-Atss18_c-o3Des(Pi_GA2)), or A10 (target: c-d6Elo(Tp_GA)), or A11 (target j-t-StCAT_p2_p-LuPXR)	Copy number ratios measured near the right border of the T-DNA using an assay targetting c-d6Elo(Pp_GA)	Most likely number of loci containing one or more T-DNA copies, tested using Chi-Square test
LBEDTZ	084:001:111:002:050:000:000:000:000:000:000:000:000	A10: 085:000:001:111:002:048:002:000:000:000:000:000:000	084:000:111:000:052:001:000:000:000:000:000:000:000	single locus
LBEDUA				double locus
LBEDWU	023:071:098:043:014:000:000:000:000:000:000:000:000	A10: 023:075:098:048:004:000:000:000:000:000:000:000:000	023:071:099:045:011:000:000:000:000:000:000:000:000	double locus
LBEFME	011:025:039:062:040:041:023:005:001:000:000:001:000	A11: 010:024:043:063:073:027:004:001:001:000:000:000:000	059:137:052:000:000:000:000:000:000:000:000:000:000	triple locus
LBEEIL	075:020:044:051:039:018:001:000:000:000:000:000:000	A04: 073:020:046:050:046:010:001:000:000:000:000:000:000	075:020:043:052:039:017:002:000:000:000:000:000:000	triple locus
LBEEBO	019:072:090:055:012:000:000:000:000:000:000:000:000	A10: 019:073:090:052:015:000:000:000:000:000:000:000:000	063:118:059:007:000:000:000:000:000:000:000:000:000	double locus
LBERLW	012:039:057:058:050:023:010:000:000:000:000:000:000	A10: 010:046:065:034:009:000:000:000:000:000:000:000:000	011:072:096:054:014:000:000:000:000:000:000:000:000	double locus

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Table 126: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

	Copy number assays targeting the T-DNA of VC-LTM595-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA)near the right T-DNA border. Copy number results obtained on the T0 plants are indicated in parentheses. Homozygosity of all plants per event was indicated if the average result of the selected T1 plants was about two fold higher than the T0 generation.													
Event	c-AHAS	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-i-Atss2_c-d4Des(Pl_GA)-195R	j-p-LuPXR_i-Atss15	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Ot febit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
LBDGCA (n=94)	1.5 (T0: 1)	3.2 (T0: 2)	1.6 (T0: 1.4)	(T0: 1)	(T0: 1.2)	1.7 (T0: 0.9)	1.6	1.6 (T0: 1)	(T0: 1.2)	1.7 (T0: 1)	(T0: 1.1)	(T0: 1.1)	3.2 (T0: 2)	1.6 (T0: 1)
LBDGBP (n=20)	(T0: 1.1)	(T0: 2.1)	(T0: 1.4)	(T0: 1.2)	(T0: 1.3)	(T0: 1.1)		(T0: 1.1)	(T0: 1.3)	(T0: 1)	(T0: 1)	(T0: 1.2)	(T0: 2.1)	(T0: 1)
LBDIHN (n=114)	1.6 (T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.7)	(T0: 1.1)	(T0: 1.2)	1.7 (T0: 1)	1.6	1.6 (T0: 1.1)		1.7 (T0: 1)	(T0: 0.9)	(T0: 1.2)	3.3 (T0: 2.2)	1.6 (T0: 1.1)

Table 127: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that were measured per event.

event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBEDTZ (n=34)	4.9 ± 0.2	0.2 ± 0	0 ± 0	2.4 ± 0.2	28 ± 1.7	31.1 ± 2.2	1.3 ± 0.2	4.9 ± 0.5	4.9 ± 2	0.8 ± 0.3	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0.1	0.1 ± 0	3.9 ± 0.8	1.5 ± 0.5	2.4 ± 0.4	4.7 ± 1.6	0.4 ± 0	0 ± 0	0.9 ± 0.1	1.8 ± 0.6	0.8 ± 0.1	1.7 ± 0.6	1.2 ± 0.3	0.2 ± 0.1
LBEDUA (n=4)	7.7 ± 0.2	0.4 ± 0.1	0 ± 0	3.6 ± 0.5	24.3 ± 0.7	32.6 ± 1	0.9 ± 0.1	4.1 ± 0.4	2.4 ± 0.2	0.3 ± 0	1.1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.9 ± 0.6	1.4 ± 0.1	2.2 ± 0.2	6.5 ± 0.3	0.5 ± 0	0 ± 0	1.1 ± 0	1.8 ± 0.2	0.7 ± 0.1	2.5 ± 0.1	0.8 ± 0.1	0.1 ± 0
LBEDWU (n=6)	6.4 ± 0.4	0.3 ± 0.1	0 ± 0	5.1 ± 0.9	26.4 ± 3.4	31.5 ± 1.2	0.8 ± 0.2	4.4 ± 0.2	2.1 ± 0.5	0.3 ± 0.1	1.4 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.2 ± 0.1	3.3 ± 0.3	1.4 ± 0.2	1.7 ± 0.2	6.1 ± 1.4	0.6 ± 0.1	0 ± 0	1 ± 0.2	1.8 ± 0.4	0.6 ± 0.2	2.6 ± 0.5	0.9 ± 0.2	0.1 ± 0

Table 128: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. For each event, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Events	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LBEDTZ (n=1)	4.9	0.2	0.0	2.5	26.4	27.0	1.3	5.6	2.2	0.3	0.8	0.7	0.1	0.1	4.3	2.4	2.0	8.5	0.3	0.0	0.9	3.2	0.9	3.3	1.7	0.3
LBEDUA (n=1)	8.0	0.4	0.0	3.9	23.6	32.3	0.9	4.2	2.1	0.2	1.2	0.7	0.1	0.1	3.9	1.5	2.2	6.7	0.5	0.0	1.1	2.0	0.7	2.7	0.9	0.1
LBEDWU (n=1)	6.1	0.3	0.0	4.9	22.2	32.8	0.8	4.8	1.9	0.3	1.2	0.6	0.1	0.1	3.0	1.4	1.7	7.7	0.5	0.0	1.3	2.5	0.9	3.4	1.2	0.1

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Table 129: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM595-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Events	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBEDTZ (n=34)	46.7 ± 3.7	8.9 ± 0.5	7.8 ± 0.6	7.9 ± 0.4	8.8 ± 0.7	3 ± 0	4 ± 0	5 ± 0	9 ± 0	5.2 ± 0.6	102.6 ± 7.1	3.4 ± 1.2	3.7 ± 1.1	36.3	30.5
LBEDUA (n=4)	60.8 ± 2.6	5.3 ± 1.5	8 ± 0	3.8 ± 0.5	5.5 ± 0.6	3 ± 0	5 ± 0	5 ± 0	6.3 ± 0.5	4.5 ± 0.6	83.8 ± 2.5	3.7 ± 0	8 ± 0.8	32.4	30.9
LBEDWU (n=6)	47.2 ± 2.7	6 ± 0	8 ± 0	3.5 ± 0.8	5.8 ± 0.4	3 ± 0	4 ± 0	4.8 ± 0.4	6.7 ± 1.8	4.3 ± 0.5	71.7 ± 12.1	2.5 ± 0.3	6.7 ± 2.1	33.8	30.0

Example 18: Plants containing the T-DNA of plasmid VC-LTM593-1qcz rc for production of EPA and DHA in seeds

All genetic elements required for EPA and DHA synthesis described in this example, were transferred on a single T-DNA using a BiBAC plasmid into the plant genome. To this end, the plasmid VC-LTM593-1qcz rc where cloned into agrobacteria, and plant tissue was incubated according to example 6 with this agrobacterial culture. Due to the selectable herbicide resistance marker, regenerated plants contained the T-DNA of VC-LTM593-1qcz rc . The genetic elements of VC-LTM593-1qcz rc and the function of each element are listed in Table 11. For convenience, all enzymes expressed in seeds of plants carrying both T-DNA of VC-LTM593-1qcz rc that are required for EPA and DHA synthesis are additionally listed Table 130.

Table 130: List of genes essential of EPA and DHA synthesis carried by the T-DNA of plasmid VC-LTM593-1qcz rc .

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
c-d12Des(Ps_GA2)	1197	Delta-12 desaturase from *Phythophthora sojae*
c-d6Des(Ot_febit)	1371	Delta-6 desaturase from *Ostreococcus tauri*
c-d6Elo(Pp_GA2)	873	Delta-6 elongase from *Physcomitrella patens*
c-d6Elo(Tp_GA2)	819	Delta-6 elongase from *Thalassiosira pseudonana*
2 copies of c-d5Des(Tc_GA2)	1320	Delta-5 desaturase from *Thraustochytrium* sp. ATCC21685
c-o3Des(Pi_GA2)	1086	Omega-3-desaturase from *Phythophthora infestans*
2 copies of c-o3Des(Pir_GA)	1092	Omega-3 desaturase from *Pythium irregulare*
c-d5Elo(Ot_GA3)	903	Delta-5 elongase from *Ostreococcus tauri*
c-d4Des(Pl_GA)2	1338	Delta-4 desaturase from *Pavlova lutheri*
c-d4Des(Tc_GA3)	1560	Delta-4 desaturase from

Genes encoding enzmyes for EPA and DHA synthesis	Length (bp)	Enzymatic function and source of encoded protein
		Thraustochytrium sp.

Fatty acid Profile in selected T1 seed batches

Fatty acid profiles, copy number measurements, and phenotypic observations of T0 plants carrying T-DNAs of plasmid VC-LTM593-1qcz rc cultivated in greenhouses during summer

One observation from the data on Table 131 was that there was a higher number of insertion events obtained from VC-LTP593-1qcz than obtained from the BiBAC constructs in examples 16 or 17. The data on Table 132 and Table 133 indicate that with respect to VLC-PUFA accumulation, in particular EPA and DHA, double copy events accumulated more than single copy events and that triple copy events accumulated more than double copy events, with accumulation for the triple copy events being approximately eight percent of total fatty acids (EPA and DHA combined, with 1.6% accumulation of total fatty acids being DHA). The highest amount accumulated was approximately fifteen percent of the total fatty acid content in the seed being EPA and DHA combined, with 3 percent of the total seed fatty acid content being DHA, see Table 133. The aerial phenotype of this construct in the T0 plant and T1 seed showed less variation than that seen in examples 16 or 17.

Table 131: Copy number measurement of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column; sc: all T1 plants where the average of all copy number assays listed in this table except the assay c-d5Des(Tc_GA) was 0.51-1.49, dc: all T1 plants where the average of all copy number assays listed in this table was 1.51-2.49, tc: all T1 plants where the average of all copy number assays listed in this table was 2.51-3.49.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pi_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-BnSETL-v1_c-o3Des(Plr_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Offebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)
sc (n=275)	1.1	1.2	1.1	1.1	1.0	2.1	1.1			1.1	1.1	1.0	2.1	1.1
dc (n=49)	1.7	1.6	1.8	1.7	1.6	3.5	1.9			1.9	2.3	1.8	3.5	1.7
tc (n=11)	2.6		3.0	3.2	2.5	5.3	3.0			2.9	3.8	2.6	5.3	2.8

Table 132: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=275)	5.1 ± 0.5	0.3 ± 0.1	0.1 ± 0.1	3.2 ± 0.5	41.3 ± 5.3	29.6 ± 2.9	0.4 ± 0.2	6 ± 0.9	0.8 ± 0.4	0.1 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	0.4 ± 0.2	0.1 ± 0.1	2 ± 0.5	1.2 ± 0.3	1.4 ± 0.5	3.8 ± 1.2	0.3 ± 0.1	0 ± 0	0 ± 0.1	0.9 ± 0.3	0 ± 0	0.9 ± 0.3	0.1 ± 0.1	0.2 ± 0.1
dc (n=49)	5.3 ± 0.6	0.3 ± 0.1	0 ± 0	3.5 ± 0.5	34.4 ± 5.9	31 ± 2.6	0.5 ± 0.2	5.5 ± 1.1	1.5 ± 0.5	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.3	0.1 ± 0.1	2.7 ± 0.8	1.5 ± 0.5	2.2 ± 1.4	5.9 ± 2.4	0.3 ± 0	0 ± 0	0.1 ± 0.1	1.3 ± 0.4	0 ± 0	1.2 ± 0.5	0.2 ± 0.2	0.2 ± 0.1
tc (n=11)	5.6 ± 0.6	0.3 ± 0.1	0 ± 0.1	3.7 ± 0.6	28 ± 5.6	31.6 ± 2.1	0.9 ± 0.4	5.1 ± 0.5	2.2 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.3 ± 0.2	0.1 ± 0.1	4.7 ± 1.7	2.8 ± 1.3	1.7 ± 1.2	6.6 ± 3.7	0.3 ± 0.1	0 ± 0	0.2 ± 0.2	1.5 ± 0.7	0 ± 0	1.6 ± 0.9	0.6 ± 0.9	0.3 ± 0.1

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Table 133: Fatty acid profiles of T1 seeds harvested from T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. For each category, the fatty acid profile of the plant/event having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	4.7	0.2	0.2	3.2	25.1	36.0	0.6	5.0	2.2	0.3	0.7	0.6	0.4	0.1	2.6	1.3	4.1	8.6	0.2	0.0	0.2	1.8	0.0	1.6	0.0	0.2
dc (n=1)	5.4	0.2	0.0	3.1	23.8	29.4	0.5	4.9	1.8	0.2	0.7	0.7	0.8	0.1	3.5	2.2	4.1	12.3	0.3	0.0	0.2	2.7	0.0	2.4	0.1	0.5
tc (n=1)	5.7	0.2	0.1	3.7	21.5	32.7	0.6	4.8	1.9	0.3	0.9	0.6	0.9	0.4	1.8	1.3	3.0	12.4	0.4	0.0	0.5	2.6	0.1	3.2	0.0	0.4

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Table 134: Phenotypic rating of T0 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. Considering each event was in this generation represented by only 1 plant, all events have been grouped into the categories indicated in the first column as described in Table 131. The number of T0 plants/events fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), Protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
sc (n=275)	56.5 ± 6.1	9 ± 0	8.6 ± 1.1	9 ± 0	5 ± 4.1	3 ± 0	4.9 ± 0.5	5 ± 0	4.8 ± 2.9	5 ± 1.1	123.9 ± 8.9	4.6 ± 0.6	1.8 ± 1.7	36.5 ± 1.1	29.1 ± 0.8
dc (n=49)	57.4 ± 6	8.9 ± 1	8.4 ± 1.4	8.9 ± 0.5	4.3 ± 2.7	3 ± 0	4.8 ± 0.8	5 ± 0	4.3 ± 2.7	5 ± 1.2	119.1 ± 15.7	4.5 ± 0.6	1.6 ± 1.2	36.2 ± 1.5	28.7 ± 1.2
tc (n=11)	60.3 ± 5.5	9 ± 0	8.5 ± 1	9 ± 0	3.5 ± 2.2	3 ± 0	5 ± 0	5 ± 0	3.5 ± 2.2	4.8 ± 1.5	125.9 ± 7	4.4 ± 0.6	3.5 ± 2.9		

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in greenhouses during winter

Specific events were examined further for copy number and displayed a variation in insertion number for the T-DNA from single insertion to partial double insertions along with double insertions. Additionally there were some variations in gene copy number (corresponding to the partial insertions and possible deletions), see Table 135, Table 136 and Table 137. The fatty acid profile data shown on Table 138 and Table 139 indicate an upper range of accumulation of combined EPA and DHA of eighteen percent of the total seed fatty acid content (event LBFDAU). In the event LBFDAU the percent of total seed fatty acid content being EPA is 15% and total seed fatty acid content being DHA is 3% in the T1. LBFDAU was analysed with a copy number indicative of a partial double copy. Another example of specific events having higher levels of EPA and DHA was LBFGKN with approximately 12 percent of the total seed fatty acid content being EPA and DHA, with 10 percent of the total seed fatty acid content being EPA and 2% being DHA. The T1 generation LBFGKN had only a single copy insertion event for VC-LTM593-1qcz rc, though data on Table 140, Table 141 and Table 142 indicate that double copy double locus events tended to accumulate more EPA and DHA combined than other copy and locus numbers with respect to the T2 seed fatty acid profile. This observation likely reflects the nature of insertion site effects and the various factors that affect the generation of elite events. Table 142 indicates that with respect to the aerial phenotype of the plants there was a range of flowering times, as indicated by DFF (days to the first flower) from 36-48. Event LBFDAU did not vary significantly from the majority of other events with a DFF value of 43, thus showing no significant effect on the aerial phenotype or significant impact on total oil or protein accumulation in the seed in the T1 plant and T2 seed.

Table 135: Copy number measurement of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from 250 segregating T1 seedlings, using zygocity analysis as illustrated in Table 137, keeping only plants that are homozygous for the desired number of loci (which are indicated in the last column of Table 137). A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygocity was indicated if the average result of the selected T1 plants was about two fold higher than the the result observed in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHA5 located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pl_GA2)	j-p-BnSETL-v1_c-o3Des(Plr_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	Conclusion from individual assays: number of T-DNA copies inserted into the genome
LBFDGG (n=50)	2.1 (T0: 1.1)	2	2 (T0: 1.3)	1.8 (T0: 1.1)	(T0: 1)	4.2 (T0: 2.2)	2 (T0: 1.1)	1.9	2	2.1 (T0: 1.1)	2.2 (T0: 1)	2.1 (T0: 1)	4.2 (T0: 2.2)	1.9 (T0: 1)	single copy
LBFGKN (n=50)	2.1 (T0: 1)	2.2	2.1 (T0: 1.3)	2.2 (T0: 1.1)	(T0: 0.8)	4.2 (T0: 2)	2.2 (T0: 1.2)	2.1	2.1	2.1 (T0: 1)	2.2 (T0: 1)	2.1 (T0: 1.1)	4.2 (T0: 2)	1.9 (T0: 1.1)	single copy
LBFIHE (n=34)	2 (T0: 1)	2.2	2.1 (T0: 1.1)	2.1 (T0: 1.2)	(T0: 1)	4.1 (T0: 1.9)	2.2 (T0: 1.1)	2.3	2.3	2.1 (T0: 1)	2.1 (T0: 1.2)	2.3 (T0: 1)	4.1 (T0: 1.9)	2.1 (T0: 1.1)	single copy
LBFLDI (n=60)	2.5 (T0: 1)	2.3	2.4 (T0: 1.1)	2.4 (T0: 1)	(T0: 1)	4.4 (T0: 1.9)	2.2 (T0: 1.2)	2.3	2.4	2.3 (T0: 0.8)	2.3 (T0: 1)	2.3 (T0: 0.9)	4.4 (T0: 1.9)	2.5 (T0: 1)	single copy
LBFPNF (n=52)	1.9 (T0: 1.1)	2.1	2.1 (T0: 1.1)	2.1 (T0: 1.3)	(T0: 1)	5.6 (T0: 2.8)	1.9 (T0: 1)	2.1	2	2 (T0: 1.2)	4 (T0: 1.9)	4.1 (T0: 1.7)	5.6 (T0: 2.8)	4.2 (T0: 1.9)	partial double copy
LBFNSQ (n=51)	2 (T0: 1.1)	2	1.9 (T0: 1.2)	1.9 (T0: 1.1)	(T0: 1.6)	7.1 (T0: 3.1)	3.8 (T0: 1.9)	4	3.9	3.7 (T0: 1.9)	4.1 (T0: 1.9)	3.8 (T0: 1.9)	7.1 (T0: 3.1)	2 (T0: 0.7)	partial double copy
LBFDGL (n=57)	2.1 (T0: 1)	2.2	3.9 (T0: 2)	3.9 (T0: 1.7)	(T0: 1.9)	6.7 (T0: 2.9)	3.8 (T0: 2)	3.8	4.1	3.8 (T0: 1.7)	4 (T0: 1.9)	3.8 (T0: 1.4)	6.7 (T0: 2.9)	1.8 (T0: 0.7)	partial double copy

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LBFIEF (n=6)	4.2 (T0: 2.5)	3.7	3.6 (T0: 2.7)	3.7 (T0: 2.7)	(T0: 2)	6.4 (T0: 3.5)	3.8 (T0: 2.3)	4.2	4.1	3.6 (T0: 2.3)	3.8 (T0: 2.6)	3.7 (T0: 1.6)	6.4 (T0: 3.5)	2.1 (T0: 0.8)	partial double copy
LBFBAV (n=50)	2 (T0: 1.1)	2.2 (T0: 1.3)	3.7 (T0: 1.5)	4 (T0: 1.7)	(T0: 1.4)	8.2 (T0: 3.7)	3.8 (T0: 1.8)	3.9	3.9	4 (T0: 1.9)	3.7 (T0: 2)	4.1 (T0: 1.7)	8.2 (T0: 3.7)	6.3 (T0: 2.9)	partial double copy
LBFPNC (n=32)	2.1 (T0: 1.1)	2	2.3 (T0: 1.2)	2 (T0: 1)	(T0: 1.4)	7.5 (T0: 3.4)	4.2 (T0: 1.7)	4	3.8	3.8 (T0: 1.8)	4.1 (T0: 1.8)	4.2 (T0: 1.4)	7.5 (T0: 3.4)	4 (T0: 1.9)	partial double copy
LBFGHQ (n=46)	3.6 (T0: 1.8)	3.8	4.1 (T0: 1.8)	4 (T0: 2.2)	(T0: 1.7)	6.1 (T0: 3.1)	3.8 (T0: 1.7)	3.8	3.7	2.1 (T0: 1.1)	2 (T0: 1.1)	2 (T0: 1.1)	6.1 (T0: 3.1)	2 (T0: 1)	partial double copy
LBFAZB (n=49)	3.9 (T0: 1.8)	4 (T0: 1.7)	3.9 (T0: 1.8)	3.9 (T0: 1.7)	(T0: 1.3)	6.6 (T0: 3.1)	3.7 (T0: 1.8)	4.1	4	3.8 (T0: 1.7)	4 (T0: 1.8)	3.7 (T0: 1.3)	6.6 (T0: 3.1)	1.9 (T0: 0.6)	partial double copy
LBFGKW (n=72)	3.8 (T0: 1.8)	3.8	4.2 (T0: 2.1)	4.1 (T0: 1.9)	(T0: 1.6)	7 (T0: 3.2)	3.9 (T0: 2)	4	4	4 (T0: 1.7)	3.6 (T0: 1.8)	3.7 (T0: 1.5)	7 (T0: 3.2)	1.7 (T0: 0.6)	partial double copy
LBFNRU (n=58)	3.8 (T0: 1.9)	4	3.8 (T0: 1.9)	3.7 (T0: 2)	(T0: 1.7)	7 (T0: 3.2)	4 (T0: 1.7)	3.7	3.9	3.8 (T0: 1.7)	4.2 (T0: 1.9)	3.8 (T0: 1.6)	7 (T0: 3.2)	1.9 (T0: 0.8)	partial double copy
LBFGIZ (n=43)	3.8 (T0: 1.9)	4.1	3.9 (T0: 1.9)	3.8 (T0: 2.1)	(T0: 1.6)	7 (T0: 2.8)	4.2 (T0: 1.8)	3.8	3.9	3.8 (T0: 1.7)	4.3 (T0: 1.5)	3.8 (T0: 1.5)	7 (T0: 2.8)	1.9 (T0: 0.8)	partial double copy
LBFIGM (n=56)	4.1 (T0: 2)	3.9	3.8 (T0: 2)	3.8 (T0: 1.7)	4 (T0: 1.5)	7.6 (T0: 2.8)	3.9 (T0: 1.7)	3.9	3.8	3.8 (T0: 1.8)	4.2 (T0: 2.2)	4.1 (T0: 1.6)	7.6 (T0: 2.8)	1.9 (T0: 0.6)	partial double copy
LBFNRR (n=61)	3.7 (T0: 1.9)	4.2	4 (T0: 2.2)	3.8 (T0: 2)	(T0: 1.7)	7.1 (T0: 2.8)	4.3 (T0: 2)	3.8	4.1	3.9 (T0: 1.7)	4.3 (T0: 1.6)	3.9 (T0: 1.6)	7.1 (T0: 2.8)	1.9 (T0: 0.7)	partial double copy
LBFNTK (n=69)	4.1 (T0: 1.9)	4.1	3.9 (T0: 2.1)	3.9 (T0: 2)	4 (T0: 1.7)	7.6 (T0: 2.9)	4 (T0: 1.8)	3.9	4.1	3.8 (T0: 1.7)	4.3 (T0: 1.9)	4.1 (T0: 1.5)	7.6 (T0: 2.9)	1.9 (T0: 0.7)	partial double copy
LBFGJA (n=42)	3.7 (T0: 1.6)	3.7	3.9 (T0: 2.1)	3.6 (T0: 2.1)	(T0: 1.7)	7.6 (T0: 3.6)	3.4 (T0: 1.7)	3.8	3.7	4.1 (T0: 1.9)	3.9 (T0: 1.9)	3.6 (T0: 1.9)	7.6 (T0: 3.6)	3.9 (T0: 2.1)	double copy
LBFIFV (n=58)	3.9 (T0: 1.7)	4	3.9 (T0: 2.4)	1.9 (T0: 1)	(T0: 1.8)	7.7 (T0: 3.9)	3.9 (T0: 1.8)	3.9	4	3.9 (T0: 2)	4 (T0: 2.6)	4.2 (T0: 1.8)	7.7 (T0: 3.9)	3.9 (T0: 2)	double copy
LBFLER (n=52)	3.8 (T0: 1.9)	3.9	3.9 (T0: 2.3)	3.8 (T0: 1.9)	(T0: 1.7)	7.7 (T0: 3.3)	3.9 (T0: 2.2)	4	4	4.1 (T0: 1.8)	4.2 (T0: 2.3)	4 (T0: 1.6)	7.7 (T0: 3.3)	3.8 (T0: 1.9)	double copy
LBFLDL (n=44)	3.8 (T0: 1.7)	3.9	3.8 (T0: 2)	3.8 (T0: 1.8)	(T0: 1.8)	7.8 (T0: 3.5)	3.9 (T0: 2.1)	3.8	4.1	4.1 (T0: 1.8)	4.1 (T0: 2.1)	3.9 (T0: 1.6)	7.8 (T0: 3.5)	3.8 (T0: 1.9)	double copy
LBFNQW (n=51)	3.8 (T0: 1.8)	3.9	3.9 (T0: 1.9)	3.9 (T0: 1.9)	(T0: 1.6)	7.9 (T0: 3.4)	3.9 (T0: 1.8)	3.9	4	4.2 (T0: 1.6)	4.2 (T0: 2)	3.9 (T0: 1.9)	7.9 (T0: 3.4)	3.9 (T0: 1.8)	double copy
LBFBAP (n=19)	2 (T0: 1.1)	2 (T0: 1.2)	2 (T0: 1)	1.8 (T0: 1.3)	(T0: 1.1)	7.5 (T0: 2.8)	3.8 (T0: 1.8)	3.9	3.8	5.7 (T0: 2.3)	5.5 (T0: 2.3)	5.7 (T0: 1.8)	7.5 (T0: 2.8)	4 (T0: 2.4)	partial triple copy
LBFDAU (n=10)	2 (T0: 1.1)	2	3.8 (T0: 1.9)	3.8 (T0: 1.8)	(T0: 1.5)	7.3 (T0: 3.7)	3.8 (T0: 2)	3.9	3.9	3.7 (T0: 1.8)	3.9 (T0: 2.4)	4.1 (T0: 1.7)	7.3 (T0: 3.7)	3.7 (T0: 1.9)	partial double copy

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LBFPRA (n=16)	2.1 (T0: 2)	2	6 (T0: 3)	1.8 (T0: 1.8)	(T0: 2)	7.4 (T0: 4.7)	3.8 (T0: 2.8)	4.1	3.7	3.7 (T0: 2.4)	3.9 (T0: 3.1)	3.7 (T0: 2.3)	7.4 (T0: 4.7)	3.8 (T0: 2.8)	partial double copy
LBFIFU (n=11)	2.2 (T0: 2)	2.5	2.4 (T0: 2.2)	2.4 (T0: 2.1)	(T0: 1.7)	6.5 (T0: 4.2)	2.6 (T0: 1.9)	3.2	4.9	4.3 (T0: 2.3)	4.2	4 (T0: 2.6)	6.5 (T0: 4.2)	3.8 (T0: 3)	partial double copy
LBFDKD (n=2)	3.6 (T0: 1.6)	3.7	3.6 (T0: 2.1)	3.9 (T0: 2.2)	(T0: 1.8)	7.6 (T0: 3.4)	2.9 (T0: 2.1)	3.5	3.6	3.8 (T0: 1.9)	4.3 (T0: 2)	4.3 (T0: 1.8)	7.6 (T0: 3.4)	4 (T0: 1.6)	double copy
LBFDJG (n=12)	3.6 (T0: 1.6)	3.7	4 (T0: 1.8)	3.7 (T0: 1.6)	(T0: 1.9)	8 (T0: 3.6)	3.6 (T0: 2)	4	3.9	4.1 (T0: 1.9)	4.1 (T0: 2.2)	3.7 (T0: 1.9)	8 (T0: 3.6)	4.5 (T0: 1.9)	double copy
LBFLFK (n=15)	3.7 (T0: 2)	3.9	3.7 (T0: 2.4)	3.6 (T0: 2.3)	(T0: 2)	7.5 (T0: 5.2)	4.1 (T0: 2.2)	3.7	3.8	3.8 (T0: 1.3)	4.1 (T0: 2.4)	3.9 (T0: 1.8)	7.5 (T0: 5.2)	4 (T0: 2)	double copy
LBFLCG (n=15)	3.8 (T0: 1.9)	3.9	3.7 (T0: 2.3)	3.7 (T0: 1.9)	(T0: 1.7)	9 (T0: 5.1)	3.8 (T0: 1.9)	3.9	3.9	3.7 (T0: 2)	3.9 (T0: 2.5)	5.6 (T0: 2.2)	9 (T0: 5.1)	3.9 (T0: 2)	double copy
LBFPQM (n=12)	4 (T0: 2)	3.8	4.1 (T0: 1.8)	4.1 (T0: 2)	(T0: 1.5)	7.6 (T0: 3.7)	3.9 (T0: 2)	4	3.8	3.8 (T0: 1.8)	4 (T0: 2)	4.2 (T0: 1.5)	7.6 (T0: 3.7)	4.1 (T0: 1.8)	double copy
LBFDHG (n=5)	4.2 (T0: 1.9)	4	3.8 (T0: 2.3)	3.8 (T0: 1.8)	(T0: 1.6)	7.7 (T0: 3.3)	3.9 (T0: 1.9)	3.7	3.6	3.8 (T0: 2)	3.9 (T0: 2.3)	3.9 (T0: 1.7)	7.7 (T0: 3.3)	3.9 (T0: 1.9)	double copy
LBFCYO (n=0)	(T0: 3)		(T0: 2.2)	(T0: 2)	(T0: 2)	(T0: 3)	(T0: 3.4)			(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 3)	(T0: 0.6)	partial double copy
LBFBAJ (n=0)	(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 1)	(T0: 1.7)	(T0: 4.8)	(T0: 2)			(T0: 2)	(T0: 2.5)	(T0: 1.8)	(T0: 4.8)	(T0: 2)	partial double copy
LBFDJI (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 3)	(T0: 2)			(T0: 1.5)	(T0: 2.5)	(T0: 2.1)	(T0: 3)	(T0: 1.7)	partial double copy
LBFGGO (n=0)	(T0: 1)		(T0: 1)	(T0: 1.1)	(T0: 1)	(T0: 2.6)	(T0: 1)			(T0: 2.1)	(T0: 2.1)	(T0: 2)	(T0: 2.6)	(T0: 1.9)	partial double copy
LBFLFP (n=0)	(T0: 0.9)		(T0: 1.2)	(T0: 1.2)	(T0: 1.8)	(T0: 3.5)	(T0: 2)			(T0: 1.8)	(T0: 2.1)	(T0: 1.7)	(T0: 3.5)	(T0: 1.7)	partial double copy
LBFNQV (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 2.7)	(T0: 1.8)			(T0: 1.7)	(T0: 2.3)	(T0: 1.7)	(T0: 2.7)	(T0: 2)	partial double copy
LBFNLT (n=0)	(T0: 1.1)		(T0: 1.2)	(T0: 1)	(T0: 1.8)	(T0: 3.3)	(T0: 1.8)			(T0: 1.8)	(T0: 2.2)	(T0: 1.8)	(T0: 3.3)	(T0: 1.9)	partial double copy
LBFLGC (n=0)	(T0: 1)		(T0: 1.1)	(T0: 1.1)	(T0: 1)	(T0: 2.5)	(T0: 1.1)			(T0: 3)	(T0: 2.6)	(T0: 2.2)	(T0: 2.5)	(T0: 1.8)	partial double copy
LBFLCW (n=0)	(T0: 0.9)		(T0: 1.2)	(T0: 1)	(T0: 0.8)	(T0: 3.6)	(T0: 1.7)			(T0: 1.5)	(T0: 2)	(T0: 1.6)	(T0: 3.6)	(T0: 1.5)	partial double copy
LBFZPJ (n=0)	(T0: 2)		(T0: 1.8)	(T0: 1.9)	(T0: 1)	(T0: 3.5)	(T0: 1)			(T0: 2.3)	(T0: 3)	(T0: 2.4)	(T0: 3.5)	(T0: 2.4)	partial double copy

LBFN5S (n=0)	(T0: 1.1)		(T0: 1)	(T0: 1.2)	(T0: 1.1)	(T0: 2.5)	(T0: 1.8)			(T0: 2.3)	(T0: 2.5)	(T0: 2)	(T0: 2.5)	(T0: 2)	partial double copy
LBGAOR (n=0)	(T0: 1.1)		(T0: 1.8)	(T0: 2)	(T0: 1.7)	(T0: 2.9)	(T0: 1.8)			(T0: 1.8)	(T0: 1.1)	(T0: 1)	(T0: 2.9)	(T0: 1.1)	partial double copy
LBFZOE (n=0)	(T0: 1)		(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 2.8)	(T0: 1)			(T0: 1.1)	(T0: 2.1)	(T0: 1.7)	(T0: 2.8)	(T0: 1.8)	partial double copy
LBFDJS (n=0)	(T0: 1)		(T0: 1.2)	(T0: 1)	(T0: 1)	(T0: 2.9)	(T0: 1.2)			(T0: 0.9)	(T0: 2.2)	(T0: 1.9)	(T0: 2.9)	(T0: 1.9)	partial double copy
LBFDKC (n=0)	(T0: 1)		(T0: 1)	(T0: 1.2)	(T0: 1.8)	(T0: 2.5)	(T0: 2.2)			(T0: 1.7)	(T0: 1.6)	(T0: 1.4)	(T0: 2.5)	(T0: 0.6)	partial double copy
LBFDKA (n=5)	4.2 (T0: 1.6)	3.8	5.4 (T0: 2.6)	5.2 (T0: 3.1)	(T0: 2.4)	10.1 (T0: 5.6)	4.8 (T0: 3.1)	6	5.7	5.7 (T0: 3.3)	5.7 (T0: 3.9)	5.9 (T0: 2.9)	10.1 (T0: 5.6)	5.7 (T0: 3.5)	partial triple copy
LBFLGH (n=0)	(T0: 0.9)		(T0: 1)	(T0: 1.1)	(T0: 1.1)	(T0: 2.9)	(T0: 2.3)			(T0: 2.5)	(T0: 3)	(T0: 2.1)	(T0: 2.9)	(T0: 2.5)	partial triple copy
LBFNUS (n=0)	(T0: 1.1)		(T0: 1)	(T0: 0.9)	(T0: 1.4)	(T0: 3.9)	(T0: 2.2)			(T0: 2.3)	(T0: 3.1)	(T0: 2.3)	(T0: 3.9)	(T0: 3.3)	partial triple copy
LBFLCH (n=0)	(T0: 1.8)		(T0: 2.1)	(T0: 2.1)	(T0: 2.3)	(T0: 5)	(T0: 3.1)			(T0: 2.3)	(T0: 5.3)	(T0: 3.3)	(T0: 5)	(T0: 3.4)	partial triple copy
LBFZMI (n=0)	(T0: 3.3)		(T0: 2.1)	(T0: 2)	(T0: 1.5)	(T0: 4.6)	(T0: 2.2)			(T0: 1.7)	(T0: 1.9)	(T0: 2.9)	(T0: 4.6)	(T0: 2.6)	partial triple copy
LBFIDT (n=7)	3.8 (T0: 1.5)	4	4.3 (T0: 2.2)	4.2 (T0: 1.9)	(T0: 1.5)	8.5 (T0: 4.5)	4 (T0: 2.5)	4.3	4.3	4.3 (T0: 2.5)	3.8 (T0: 3.8)	4.2 (T0: 2.4)	8.5 (T0: 4.5)	3.9 (T0: 2.4)	partial triple copy
LBFAZW (n=0)	(T0: 1.8)	(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 1.4)	(T0: 4.5)	(T0: 1.7)			(T0: 2.5)	(T0: 3.3)	(T0: 2.4)	(T0: 4.5)	(T0: 2.5)	partial triple copy
LBFBBI (n=0)	(T0: 2)	(T0: 2)	(T0: 1.9)	(T0: 1.5)	(T0: 1.6)	(T0: 4.2)	(T0: 1.9)			(T0: 1.9)	(T0: 2.7)	(T0: 1.9)	(T0: 4.2)	(T0: 1.7)	partial triple copy
LBFAZW (n=0)	(T0: 1.8)	(T0: 2)	(T0: 2.1)	(T0: 1.8)	(T0: 1.4)	(T0: 4.5)	(T0: 1.7)			(T0: 2.5)	(T0: 3.3)	(T0: 2.4)	(T0: 4.5)	(T0: 2.5)	partial triple copy

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Table 136: Expected Mendelian segregation of the genotype in T1 seeds for some possible T-DNA insertion scenarios. Listed are the expected copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. sc: single copy, dc: double copy

	Ratio of copy numbers (cn) expected T1 seed segregating for given locus configuration												
Locus configuration	cn=0	cn=1	cn=2	cn=3	cn=4	cn=5	cn=6	cn=7	cn=8	cn=9	cn=10	cn=11	cn=12
1 sc locus	1	2	1										
1 dc locus	1		2		1								
2 sc loci	1	4	6	4	1								
2 sc loci, 1 dc locus	1	2	3	4	3	2	1						
3 sc loci, 1 dc locus	1	4	8	12	14	12	8	4	1				

Table 137: Observed Medelian segregation of the genotype of T1 seeds of events from construct VC-LTM593-1qcz rc. The segregation has been analysed at three positions of the T-DNA. For each position, the number of seedlings have been counted that have a copy number (aritmetically rounded) of 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12. The of seedlings counted for each copy number category are separated by colon, displaying the categories in the following order: 0 : 1 : 2 : 3 : 4 : 5 : 6 : 7 : 8 : 9 : 10 : 11 : 12. Listed are the observed copy number segregation ratios for T1 seeds segregating for one or more unlinked genomic loci, which contain one or more linked copies of T-DNA insertions. The observed frequencies for each assay have been compared against expected frequencies for various locus configurations listed in Table 136 using Chi-Square analysis. The last column displays the total number of loci that are segregating in the genome of a given event. Many events contain truncated insertions, as was evident when some assays indicate single copy insertion at e.g. the left border (e.g. event LBFDAU, LPFPNC), while other positions on the T-DNA clearly indicate a double copy insertion that was either inserted in one locus (e.g. event LBFPNC), or in two loci (e.g. event LBFDAU)

Event	Copy number ratios measured near the left border of the T-DNA using assay A1	Copy number ratios measured using T-DNA internal assays targeting reagions that had copy number results indicating truncated T-DNA insertions using assay A06, or A08, or A09, or A10	Copy number ratios measured near the right border of the T-DNA using assay A12	Most likely number of loci containing one or more T-DNA

				copies, tested using Chi-Square test
LBFDGG	073 : 122 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 076 : 119 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	073 : 123 : 052 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGKN	057 : 131 : 059 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 057 : 130 : 059 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	057 : 130 : 060 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIHE	076 : 125 : 042 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 075 : 123 : 035 : 009 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	076 : 122 : 036 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFLDI	068 : 101 : 059 : 018 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 075 : 094 : 062 : 015 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 106 : 053 : 018 : 002 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFPNF	065 : 128 : 055 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 066 : 000 : 127 : 001 : 053 : 002 : 000 : 000 : 000 : 000 : 000 : 000	064 : 001 : 127 : 001 : 051 : 002 : 002 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNSQ	063 : 109 : 066 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 063 : 000 : 110 : 011 : 049 : 010 : 000 : 000 : 000 : 000 : 000 : 000	063 : 114 : 061 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFDGL	072 : 113 : 062 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 072 : 000 : 108 : 007 : 061 : 000 : 001 : 000 : 000 : 000 : 000 : 000	073 : 113 : 061 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIEF	042 : 000 : 018 : 020 : 013 : 002 : 004 : 000 : 001 : 000 : 000 : 000 : 000	A12 : 042 : 000 : 025 : 015 : 012 : 005 : 000 : 001 : 000 : 000 : 000 : 000	041 : 032 : 020 : 005 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFBAV	068 : 128 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 068 : 000 : 128 : 000 : 053 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 000 : 000 : 122 : 006 : 000 : 044 : 009 : 000 : 000 : 000 : 000 : 000	one locus
LBFPNC	043 : 074 : 035 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 043 : 002 : 072 : 001 : 034 : 001 : 000 : 000 : 000 : 000 : 000 : 000	043 : 000 : 074 : 001 : 034 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGHQ	067 : 000 : 113 : 007 : 051 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 065 : 110 : 057 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	066 : 104 : 059 : 003 : 003 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFAZB	078 : 001 : 158 : 002 : 055 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 078 : 001 : 151 : 008 : 055 : 002 : 001 : 000 : 000 : 000 : 000 : 000	078 : 161 : 054 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGKW	047 : 000 : 124 : 007 : 070 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 047 : 000 : 124 : 012 : 065 : 000 : 000 : 000 : 000 : 000 : 000 : 000	047 : 129 : 073 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus

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LBFNRU	070 : 000 : 120 : 003 : 056 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 069 : 000 : 121 : 001 : 056 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 121 : 058 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGIZ	073 : 000 : 108 : 001 : 045 : 004 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 073 : 000 : 108 : 002 : 044 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000	072 : 109 : 048 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIGM	055 : 000 : 133 : 004 : 056 : 000 : 001 : 000 : 000 : 000 : 000 : 000	A04 : 054 : 000 : 133 : 004 : 056 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	055 : 137 : 057 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNRR	070 : 000 : 116 : 004 : 057 : 000 : 000 : 000 : 001 : 000 : 000 : 000	A11 : 070 : 000 : 115 : 001 : 062 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 117 : 061 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFNTK	054 : 003 : 119 : 005 : 063 : 004 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 053 : 003 : 115 : 003 : 070 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	053 : 122 : 072 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFGJA	088 : 000 : 111 : 006 : 041 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 088 : 002 : 111 : 003 : 041 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	089 : 001 : 113 : 003 : 039 : 001 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFIFV	070 : 000 : 119 : 000 : 056 : 002 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 070 : 119 : 057 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 000 : 119 : 000 : 056 : 001 : 001 : 000 : 000 : 000 : 000 : 000	one locus
LBFLER	073 : 000 : 123 : 002 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 074 : 000 : 121 : 002 : 050 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	073 : 000 : 123 : 002 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFLDL	069 : 000 : 129 : 008 : 043 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 069 : 000 : 130 : 002 : 045 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	068 : 000 : 130 : 006 : 041 : 001 : 001 : 000 : 000 : 000 : 000 : 000	one locus
LBFNQW	070 : 001 : 123 : 007 : 045 : 002 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 070 : 000 : 123 : 001 : 053 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	070 : 001 : 122 : 005 : 047 : 003 : 000 : 000 : 000 : 000 : 000 : 000	one locus
LBFBAP	059 : 123 : 064 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 059 : 001 : 123 : 015 : 049 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	009 : 037 : 068 : 057 : 051 : 022 : 002 : 000 : 000 : 000 : 000 : 000	double locus, isolation of one locus
LBFDAU	059 : 123 : 062 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : 069 : 090 : 062 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	013 : 072 : 087 : 062 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFPRA	024 : 075 : 087 : 057 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 024 : 041 : 060 : 058 : 052 : 012 : 001 : 000 : 000 : 000 : 000 : 000 : 000	024 : 041 : 060 : 051 : 049 : 022 : 001 : 000 : 000 : 000 : 000 : 000	double locus, isolation of one locus
LBFIFU	019 : 075 : 105 : 034 : 013 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A13 : 019 : 040 : 060 : 067 : 044 : 013 : 003 : 000 : 000 : 000 : 000 : 000 : 000	019 : 040 : 060 : 072 : 039 : 013 : 003 : 000 : 000 : 000 : 000 : 000	double locus,

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	000	000 : 000	000	isolation of one locus
LBFDKD	007 : 031 : 045 : 018 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 007 : 031 : 046 : 017 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	007 : 031 : 044 : 017 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJG	026 : 078 : 088 : 036 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 029 : 072 : 085 : 035 : 017 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	026 : 076 : 077 : 037 : 022 : 004 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLFK	018 : 062 : 096 : 058 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A11 : 018 : 061 : 096 : 059 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	018 : 061 : 098 : 057 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLCG	015 : 059 : 087 : 068 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 016 : 058 : 087 : 067 : 016 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	016 : 056 : 090 : 065 : 015 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFPQM	008 : 058 : 094 : 060 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 008 : 058 : 094 : 062 : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	008 : 058 : 095 : 061 : 012 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDHG	022 : 064 : 084 : 037 : 004 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 023 : 062 : 090 : 033 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	023 : 063 : 089 : 034 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFCYO	091 : 001 : 002 : 013 : 090 : 001 : 006 : 031 : 001 : 000 : 000 : 000 : 000	A14 : 059 : 126 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	059 : 126 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFBAJ	056 : 140 : 048 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 017 : 056 : 093 : 064 : 014 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	016 : 056 : 091 : 032 : 043 : 005 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJI	151 : 285 : 171 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 051 : 073 : 118 : 157 : 149 : 036 : 023 : 002 : 001 : 000 : 000 : 000 : 000	049 : 152 : 228 : 129 : 041 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFGGO	017 : 034 : 030 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 009 : 011 : 030 : 023 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	009 : 011 : 031 : 023 : 007 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLFP	047 : 094 : 053 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 046 : 092 : 056 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	010 : 038 : 069 : 049 : 027 : 001 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNQV	174 : 289 : 138 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 174 : 286 : 138 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	049 : 163 : 210 : 126 : 048 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNLT	149 : 279 : 145 : 010 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 015 : 067 : 130 : 063 : 033 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	029 : 146 : 214 : 136 : 055 : 007 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLGC	064 : 121 : 057 : 005 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : 034 : 059 : 060 : 049 : 026 : 006 : 000 : 002 : 000 : 000 : 000 : 000	013 : 068 : 112 : 048 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFLCW	207 : 283 : 098 : 003 : 000 : 000 :	A12 : 065 : 152 : 224 : 131 : 018 :	064 : 156 : 232 : 110 : 022 : 001 :	double

	000 : 000 : 000 : 000 : 000 : 000 : 000	001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	000 : 000 : 000 : 000 : 000 : 000 : 000	locus
LBFZPJ	060 : 000 : 129 : 000 : 059 : 000 : 001 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 013 : C32 : 042 : 066 : 059 : 029 : 008 : 000 : 000 : 000 : 000 : 000 : 000	013 : 032 : 044 : 065 : 049 : 036 : 010 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFNSS	155 : 359 : 142 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 044 : 094 : 127 : 183 : 113 : 058 : 033 : 003 : 002 : 000 : 000 : 000 : 000	042 : 163 : 268 : 141 : 039 : 003 : 001 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBGAOR	149 : 302 : 144 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A04 : 040 : 158 : 274 : 103 : 019 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	161 : 310 : 120 : 003 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFZOE	026 : 060 : 035 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 010 : 025 : 051 : 026 : 008 : 002 : 000 : 000 : 000 : 000 : 000 : 000 : 000	010 : 025 : 049 : 031 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDJS	048 : 131 : 060 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 008 : 043 : 090 : 074 : 026 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	008 : 043 : 089 : 075 : 025 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	double locus
LBFDKC	001 : 109 : 133 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A07 : 001 : 000 : 109 : 008 : 126 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	001 : 108 : 132 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	no wildtypes
LBFDKA	015 : 072 : 101 : 052 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 001 : 012 : 041 : 054 : 076 : 045 : 018 : 001 : 000 : 000 : 000 : 000 : 000	001 : 012 : 041 : 053 : 069 : 050 : 019 : 003 : 000 : 000 : 000 : 000 : 000	triple locus
LBFLGH	061 : 137 : 051 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 015 : 034 : 052 : 068 : 039 : 026 : 012 : 002 : 001 : 000 : 000 : 000 : 000	014 : 033 : 053 : 060 : 045 : 030 : 012 : 001 : 000 : 000 : 000 : 000 : 000	triple locus
LBFNUS	054 : 137 : 058 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 003 : 016 : 048 : 054 : 034 : 008 : 003 : 000 : 000 : 000 : 000 : 000 : 000	004 : 020 : 071 : 081 : 060 : 010 : 003 : 000 : 000 : 000 : 000 : 000 : 000	triple locus
LBFLCH	019 : 077 : 090 : 052 : 011 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 019 : 039 : 013 : 006 : 050 : 050 : 021 : 008 : 019 : 013 : 009 : 002 : 000	019 : 039 : 013 : 041 : 063 : 027 : 026 : 013 : 007 : 000 : 000 : 000 : 000	triple locus
LBFZMI	017 : 016 : 036 : 038 : 023 : 027 : 005 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 017 : 043 : 061 : 033 : 008 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	007 : 015 : 039 : 051 : 034 : 014 : 002 : 000 : 000 : 000 : 000 : 000 : 000	triple locus
LBFIDT	069 : 000 : 131 : 000 : 045 : 001 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 015 : 033 : 058 : 064 : 048 : 015 : 012 : 002 : 000 : 000 : 000 : 000 : 000	003 : 018 : 050 : 056 : 061 : 031 : 019 : 008 : 000 : 000 : 000 : 000 : 000	triple locus, isolation of one locus
LBFAZW	013 : 065 : 109 : 041 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 004 : 021 : 073 : 086 : 040 : 010 : 001 : 000 : 000 : 000 : 000 : 000 : 000	003 : 018 : 069 : 079 : 048 : 013 : 002 : 000 : 001 : 000 : 000 : 000 : 000	trippel locus
LBFBBI	005 : 057 : 139 : 040 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 009 : 030 : 074 : 066 : 051 : 014 : 001 : 000 : 000 : 000 : 000 : 000 : 000	005 : 065 : 127 : 044 : 004 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	trippel locus

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LBFAZW	013 : 065 : 109 : 041 : 006 : 000 : 000 : 000 : 000 : 000 : 000 : 000 : 000	A12 : 004 : 021 : 073 : 086 : 040 : 010 : 001 : 000 : 000 : 000 : 000 : 000 : 000	003 : 018 : 069 : 079 : 048 : 013 : 002 : 000 : 001 : 000 : 000 : 000 : 000	trippel locus

Table 138: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDGG (n=50)	4.8 ± 0.2	0.2 ± 0	0 ± 0	2.9 ± 0.3	28.3 ± 1.5	35.6 ± 1	0.8 ± 0.1	4.5 ± 0.3	1.9 ± 0.2	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.7 ± 0.2	1.5 ± 0.1	2.2 ± 0.3	7.7 ± 0.8	0.4 ± 0	0 ± 0	0.3 ± 0.1	1.9 ± 0.2	0.2 ± 0	1.4 ± 0.3	0.3 ± 0.1	0.2 ± 0.1
LBFGKN (n=50)	4.6 ± 0.3	0.1 ± 0	0 ± 0	3 ± 0.2	28.3 ± 1.6	35.2 ± 0.7	1 ± 0.1	4.6 ± 0.3	2 ± 0.3	0.5 ± 0.2	0.8 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	2.8 ± 0.2	1.4 ± 0.1	2.3 ± 0.2	8.1 ± 0.9	0.4 ± 0	0 ± 0	0.3 ± 0	1.9 ± 0.2	0 ± 0	1.4 ± 0.3	0.2 ± 0	0.2 ± 0
LBFIHE (n=34)	5.4 ± 0.9	0.2 ± 0.1	0 ± 0	3.1 ± 0.4	26.6 ± 2.1	34.7 ± 1.4	0.9 ± 0.2	3.9 ± 0.4	2.6 ± 0.9	0.3 ± 0.1	1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.1 ± 0	3.1 ± 0.3	1.5 ± 0.2	2.5 ± 0.4	8.4 ± 1	0.4 ± 0	0 ± 0	0.3 ± 0.1	1.6 ± 0.2	0.1 ± 0	1.5 ± 0.3	0.2 ± 0.1	0.3 ± 0.1
LBFLDI (n=60)	6.6 ± 1.2	0.3 ± 0.1	0 ± 0	2.2 ± 0.2	31.1 ± 3.3	30 ± 1.9	0.7 ± 0.2	6 ± 0.6	1.1 ± 0.3	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.4 ± 0.3	0.2 ± 0.1	2.6 ± 0.2	1.6 ± 0.2	1.7 ± 0.2	8.2 ± 1.7	0.4 ± 0	0 ± 0	0.3 ± 0.1	2.4 ± 0.5	0.1 ± 0	1.4 ± 0.3	0.3 ± 0	0.4 ± 0.1
LBFPNF (n=52)	5 ± 0.2	0.2 ± 0	0 ± 0	2.5 ± 0.2	30.5 ± 1.3	27.8 ± 1.1	1.8 ± 0.2	4.5 ± 0.4	2.9 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.1 ± 0	0 ± 0	3.5 ± 0.3	1.5 ± 0.4	2.5 ± 0.2	9.8 ± 0.8	0.4 ± 0	0 ± 0	0.3 ± 0	2.7 ± 0.3	0.1 ± 0	1.2 ± 0.1	0.3 ± 0	0.2 ± 0.1
LBFNSQ (n=51)	5 ± 0.4	0.1 ± 0	0 ± 0	2.7 ± 0.5	26.9 ± 1.6	35.7 ± 1.9	0.8 ± 0.2	5.4 ± 0.6	1.5 ± 0.2	0.3 ± 0.1	0.8 ± 0.1	0.4 ± 0.4	0.2 ± 0.1	0.1 ± 0	4.3 ± 0.6	2.2 ± 0.3	1.6 ± 0.4	6.8 ± 1.4	0.4 ± 0	0 ± 0	0.4 ± 0	1.7 ± 0.3	0.1 ± 0	1.3 ± 0.3	0.6 ± 0.2	0.5 ± 0.3
LBFDGL (n=57)	4.9 ± 0.1	0.1 ± 0	0 ± 0	3 ± 0.4	26.9 ± 2.2	36.3 ± 1.9	0.6 ± 0.2	5.8 ± 0.7	1.5 ± 0.8	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.2 ± 0	0.1 ± 0	4.7 ± 0.7	3 ± 0.5	0.8 ± 0.3	5.5 ± 1.8	0.4 ± 0	0 ± 0	0.3 ± 0.1	1.4 ± 0.3	0.1 ± 0	1 ± 0.4	0.9 ± 0.3	0.2 ± 0.1
LBFIEF (n=6)	5 ± 0.2	0.1 ± 0	0 ± 0	3.4 ± 0.4	27.6 ± 2.9	36.2 ± 2.1	0.6 ± 0.1	5.7 ± 0.5	1.5 ± 0.2	0.4 ± 0.2	1 ± 0.1	0.7 ± 0	0.2 ± 0	0.1 ± 0.1	4.2 ± 0.3	2.5 ± 0.3	0.7 ± 0.3	5.2 ± 0.8	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.6 ± 0.3	0.1 ± 0	1 ± 0.3	0.9 ± 0.2	0.2 ± 0
LBFBAV (n=50)	5.1 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.3	29.5 ± 1.3	27.2 ± 1.4	1.6 ± 0.2	5.3 ± 0.3	1.6 ± 0.1	0.3 ± 0	0.8 ± 0.2	0.7 ± 0	0.1 ± 0	0.1 ± 0.1	10.9 ± 0.7	6.5 ± 0.5	0.1 ± 0.1	1.8 ± 0.7	0.4 ± 0	0 ± 0	0.1 ± 0.1	0.8 ± 0.3	0.1 ± 0.1	0.3 ± 0.1	3 ± 0.5	0.7 ± 0.1
LBFPNC (n=32)	4.8 ± 0.3	0.1 ± 0	0 ± 0	3.9 ± 0.7	26.7 ± 1.7	35.6 ± 1	0.8 ± 0.1	3.3 ± 0.5	1.7 ± 0.3	0.2 ± 0.1	1.1 ± 0.2	0.7 ± 0	0.2 ± 0	0 ± 0	4.9 ± 0.9	2 ± 0.4	2.4 ± 0.7	6.9 ± 1.6	0.4 ± 0.1	0 ± 0	0.3 ± 0.1	1.9 ± 0.5	0.1 ± 0	0.9 ± 0.2	0.4 ± 0.2	0.4 ± 0.1
LBFGHQ (n=46)	5.1 ± 0.2	0.1 ± 0.1	0 ± 0	2.4 ± 0.1	30.7 ± 1.3	35 ± 1.7	0.5 ± 0.1	7.7 ± 0.6	1.3 ± 0.2	0.4 ± 0.1	0.9 ± 0.3	0.8 ± 0.1	0.1 ± 0	0.1 ± 0	2.5 ± 0.3	1.7 ± 0.2	0.6 ± 0.1	4.9 ± 0.7	0.4 ± 0	0 ± 0	0.3 ± 0	1.9 ± 0.3	0.2 ± 0	1 ± 0.2	0.8 ± 0.1	0.3 ± 0.1
LBFAZB (n=49)	4.9 ± 0.1	0.1 ± 0	0 ± 0	3.1 ± 0.2	28.4 ± 1.6	34.1 ± 1.1	1 ± 0.1	4.7 ± 0.4	1.9 ± 0.3	0.3 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.1 ± 0.1	0.1 ± 0	6.3 ± 0.7	3.3 ± 0.3	0.8 ± 0.2	4.5 ± 0.9	0.3 ± 0	0 ± 0	0.4 ± 0	1.4 ± 0.2	0.1 ± 0	0.9 ± 0.2	1.5 ± 0.3	0.2 ± 0

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LBFGKW (n=72)	5.1 ± 0.2	0.2 ± 0	0 ± 0	2.5 ± 0.2	30.1 ± 1.3	32.1 ± 1.6	1.1 ± 0.1	6.1 ± 0.4	1.7 ± 0.3	0.2 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	6 ± 0.7	3.3 ± 0.4	0.5 ± 0.1	4.1 ± 0.8	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.5 ± 0.3	0.1 ± 0	0.8 ± 0.2	1.7 ± 0.3	0.3 ± 0.1
LBFNRU (n=58)	5 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	31.8 ± 2.5	29.2 ± 1.9	1 ± 0.1	7.1 ± 0.5	1.2 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.8 ± 0	0.1 ± 0	0.1 ± 0	5.9 ± 0.7	3.6 ± 0.5	0.4 ± 0.1	4.2 ± 0.9	0.4 ± 0	0 ± 0	0.4 ± 0	1.7 ± 0.4	0.1 ± 0	0.9 ± 0.3	1.9 ± 0.4	0.3 ± 0.1
LBFGIZ (n=43)	4.9 ± 0.6	0.2 ± 0.1	0 ± 0	2.6 ± 0.4	30.5 ± 3	30 ± 2.6	1 ± 0.2	6.2 ± 0.8	1.3 ± 0.2	0.2 ± 0	0.8 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.2 ± 0	5.7 ± 0.8	3.5 ± 0.5	0.6 ± 0.1	5.3 ± 1.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.1 ± 0.4	0.1 ± 0	1 ± 0.2	1.7 ± 0.4	0.4 ± 0.1
LBFIGM (n=56)	4.8 ± 0.4	0.2 ± 0	0 ± 0	2.3 ± 0.2	31.9 ± 2.7	30.6 ± 2.7	1 ± 0.1	6.3 ± 0.7	1.3 ± 0.2	0.2 ± 0	0.7 ± 0.1	0.8 ± 0.1	0.1 ± 0	0.1 ± 0	5 ± 0.7	2.5 ± 0.3	1 ± 0.2	5.4 ± 1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.5	0.4 ± 2.2	1 ± 0.2	1.2 ± 0.2	0.3 ± 0.1
LBFNRR (n=61)	5 ± 0.2	0.2 ± 0	0 ± 0	2.8 ± 0.4	31.4 ± 1.5	30 ± 2.1	1.1 ± 0.1	7 ± 0.6	1.3 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.8 ± 0.1	0.1 ± 0	0.1 ± 0	5.3 ± 1.2	3.5 ± 0.3	0.4 ± 0.1	4.4 ± 1.1	0.4 ± 0	0 ± 0	0.3 ± 0	1.7 ± 0.4	0 ± 0	0.8 ± 0.2	1.7 ± 0.3	0.3 ± 0.1
LBFNTK (n=69)	5.1 ± 0.4	0.2 ± 0	0 ± 0	2.5 ± 0.2	29.6 ± 1.4	29 ± 1.8	1.3 ± 0.2	6.4 ± 0.6	1.5 ± 0.3	0.1 ± 0.1	0.8 ± 0.1	0.8 ± 0	0.1 ± 0	0.1 ± 0	6.7 ± 0.8	3.8 ± 0.5	0.6 ± 0.2	5 ± 0.9	0.4 ± 0	0 ± 0	0.5 ± 0.1	2 ± 0.4	0.1 ± 0	0.9 ± 0.2	1.8 ± 0.3	0.5 ± 0.1
LBFGJA (n=42)	5.2 ± 0.2	0.2 ± 0	0 ± 0	3.1 ± 0.2	27.7 ± 0.8	34.5 ± 0.8	1.9 ± 0.2	3.3 ± 0.2	5.8 ± 0.9	0.5 ± 0.2	0.8 ± 0	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	4.7 ± 0.6	1.5 ± 0.2	1.3 ± 0.2	3.8 ± 0.6	0.3 ± 0	0 ± 0	0.7 ± 0.1	1.4 ± 0.2	0.1 ± 0	0.8 ± 0.2	1.2 ± 0.2	0.1 ± 0
LBFIFV (n=58)	4.8 ± 0.4	0.1 ± 0	0 ± 0	3.2 ± 0.3	27.6 ± 1.5	37.3 ± 0.9	0.8 ± 0.1	3.7 ± 0.2	1.8 ± 0.3	0.2 ± 0	0.9 ± 0.1	0.7 ± 0.2	0.1 ± 0	0.1 ± 0	6.3 ± 0.6	2.5 ± 0.2	1.3 ± 0.2	4.2 ± 0.7	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.1 ± 0.2	0.1 ± 0	1.1 ± 0.3	1 ± 0.2	0.2 ± 0
LBFLER (n=52)	4.7 ± 0.1	0.1 ± 0	0 ± 0	3.1 ± 0.2	28.7 ± 2	33.9 ± 1.1	1 ± 0.1	4.4 ± 0.3	1.9 ± 0.5	0.3 ± 0	0.9 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	5.9 ± 0.6	2.9 ± 0.3	1.1 ± 0.2	5.3 ± 0.7	0.4 ± 0	0 ± 0	0.5 ± 0.1	1.7 ± 0.2	0 ± 0	1 ± 0.2	1.1 ± 0.2	0.2 ± 0.1
LBFLDL (n=44)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3.4 ± 0.3	28 ± 1	34.6 ± 1	1 ± 0.1	4.2 ± 0.2	2.2 ± 0.5	0.3 ± 0	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	5.2 ± 0.6	2.5 ± 0.4	1.2 ± 0.3	5.5 ± 0.7	0.4 ± 0	0 ± 0	0.6 ± 0.1	1.9 ± 0.2	0.1 ± 0	0.8 ± 0.2	1 ± 0.2	0.2 ± 0
LBFNQW (n=51)	4.8 ± 0.2	0.1 ± 0	0 ± 0	3.2 ± 0.3	27.6 ± 1.5	33.5 ± 1.1	1 ± 0.1	4.2 ± 0.3	2 ± 0.3	0.3 ± 0	1.3 ± 0.3	0.9 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	5.3 ± 0.5	2.7 ± 0.4	1.1 ± 0.2	6.2 ± 1	0.4 ± 0	0 ± 0	0.5 ± 0.1	2 ± 0.3	0.1 ± 0	0.9 ± 0.2	0.8 ± 0.4	0.5 ± 0.1
LBFBAP (n=19)	4.8 ± 0.1	0.1 ± 0	0 ± 0	3 ± 0.2	26 ± 0.9	33.8 ± 1	1.2 ± 0.1	3.4 ± 0.2	2.4 ± 0.3	0.3 ± 0	0.8 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	7.4 ± 0.7	2.9 ± 0.3	2.1 ± 0.3	6.2 ± 0.8	0.3 ± 0	0 ± 0	0.3 ± 0	1.7 ± 0.2	0.1 ± 0	1.1 ± 0.1	0.6 ± 0.1	0.4 ± 0.1
LBFDAU (n=10)	5.2 ± 1	0.2 ± 0	0 ± 0	3.3 ± 0.3	20.5 ± 0.9	31.8 ± 0.5	0.9 ± 0.1	4.1 ± 0.2	2.5 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.6 ± 0.1	0.2 ± 0	0.1 ± 0	4.2 ± 0.2	3.1 ± 0.3	1.6 ± 0.3	13.9 ± 1	0.4 ± 0	0 ± 0	0.2 ± 0	2.3 ± 0.2	0.1 ± 0	2.6 ± 0.4	0.4 ± 0.1	0.3 ± 0
LBFPRA (n=16)	4.9 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.6	25.4 ± 2.2	31 ± 1.3	1.3 ± 0.2	5.4 ± 0.8	2 ± 0.4	0.2 ± 0.1	0.8 ± 0.1	0.8 ± 0	0.1 ± 0	0.1 ± 0	2.9 ± 0.6	1.6 ± 0.3	3 ± 0.3	12.3 ± 1.4	0.4 ± 0	0 ± 0	0.2 ± 0	2.7 ± 0.2	0 ± 0	1.4 ± 0.2	0.1 ± 0	0.4 ± 0.1
LBFIFU (n=11)	4.7 ± 0.2	0.1 ± 0	0 ± 0	3.8 ± 0.9	25.9 ± 2.6	34.7 ± 1.3	1.1 ± 0.3	3.6 ± 0.6	2.7 ± 0.9	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.2 ± 0.6	1.7 ± 0.3	2.7 ± 0.5	8 ± 1.8	0.3 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.3	0.1 ± 0.1	1.3 ± 0.4	0.3 ± 0.2	0.2 ± 0
LBFDKD (n=2)	5.1 ± 0.2	0.2 ± 0	0 ± 0	3 ± 0.9	26.1 ± 3.9	35.6 ± 0.6	0.6 ± 0	4.8 ± 0.7	1.3 ± 0.4	0.2 ± 0.1	0.9 ± 0.2	0.8 ± 0	0.3 ± 0	0.2 ± 0	5.1 ± 1.6	3 ± 1.3	0.9 ± 0.4	5.7 ± 0.7	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.2 ± 0.5	0.1 ± 0	1.1 ± 0.2	1.4 ± 0.7	0.3 ± 0
LBFDJG (n=12)	4.4 ± 0.1	0.1 ± 0	0 ± 0	3.4 ± 0.3	27.3 ± 1.4	33.1 ± 1.9	1.1 ± 0.2	3.6 ± 0.1	2.1 ± 0.4	0.4 ± 0.1	0.9 ± 0.1	0.6 ± 0.2	0.1 ± 0	0 ± 0	6.1 ± 1.1	2.6 ± 0.4	1.6 ± 0.4	6.8 ± 1.4	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.7 ± 0.2	0.1 ± 0	1.3 ± 0.4	1 ± 0.4	0.4 ± 0.1
LBFLFK (n=15)	5.1 ± 0.3	0.2 ± 0	0 ± 0	2.5 ± 0.1	28.7 ± 1.6	29 ± 1.7	1.2 ± 0.2	5.6 ± 0.4	1.5 ± 0.2	0.2 ± 0	0.8 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.8 ± 0.3	2.4 ± 0.1	1.3 ± 0.1	9.9 ± 0.8	0.4 ± 0.1	0 ± 0	0.5 ± 0	3 ± 0.3	0.1 ± 0	1.8 ± 0.2	0.6 ± 0.1	0.3 ± 0.1

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LBFLCG (n=15)	4.9 ± 0.3	0.1 ± 0	0 ± 0	3.6 ± 0.3	25.8 ± 1.4	34.1 ± 0.8	0.9 ± 0.1	4.4 ± 0.2	1.9 ± 0.2	0.3 ± 0	0.9 ± 0	0.6 ± 0	0.1 ± 0	0.1 ± 0	3.7 ± 0.5	2.2 ± 0.3	1.4 ± 0.2	9 ± 0.7	0.3 ± 0	0 ± 0	0.4 ± 0.1	2.5 ± 0.2	0.1 ± 0	1.6 ± 0.3	0.6 ± 0.2	0.2 ± 0
LBFPQM (n=12)	5.2 ± 0.3	0.1 ± 0	0 ± 0	3.8 ± 0.4	26.3 ± 1.2	31.6 ± 1	1.2 ± 0.1	4.3 ± 0.4	1.8 ± 0.2	0.2 ± 0	1.1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.2 ± 0	4.8 ± 0.6	3 ± 0.4	1.1 ± 0.2	8.2 ± 1.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.2 ± 0.2	0.1 ± 0	1.5 ± 0.2	0.8 ± 0.1	0.5 ± 0.2
LBFDHG (n=5)	5.4 ± 0.1	0.2 ± 0	0 ± 0	2.3 ± 0.2	24 ± 0.6	34 ± 0.7	1 ± 0.1	4.1 ± 0.2	2.7 ± 0.1	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	10.4 ± 0.8	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.4 ± 0.1	0.3 ± 0	2.7 ± 0.2	0.5 ± 0	0.2 ± 0
LBFDKA (n=5)	5.1 ± 0.3	0.1 ± 0	0 ± 0	3.7 ± 0.4	24.2 ± 2.8	29.7 ± 1.1	1.5 ± 0.3	4.4 ± 0.3	1.8 ± 0.1	0.2 ± 0	1.1 ± 0.1	0.9 ± 0.1	0.2 ± 0.1	0.2 ± 0	7.6 ± 0.6	5.1 ± 0.8	0.6 ± 0.1	6.6 ± 1	0.4 ± 0	0 ± 0	0.3 ± 0.1	2 ± 0.3	0.1 ± 0	1.3 ± 0.5	1.8 ± 0.2	0.9 ± 0
LBFIDT (n=7)	5 ± 0.1	0.2 ± 0	0 ± 0	2.6 ± 0.1	27 ± 1.7	30.8 ± 1.2	1.1 ± 0.2	6.2 ± 0.4	1.8 ± 0.2	0.4 ± 0.1	0.8 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.5 ± 1.3	3 ± 0.9	1 ± 0.2	8.2 ± 0.6	0.4 ± 0	0 ± 0	0.5 ± 0.1	2.6 ± 0.2	0.1 ± 0	1.7 ± 0.2	1 ± 0.3	0.3 ± 0.1

Table 139: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1n-7	16:3n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3n-3	18:3n-6	18:4n-3	20:0	20:1n-9	20:2n-6	20:3n-3	20:3n-6	20:4n-3	20:4n-6	20:5n-3	22:0	22:1n-9	22:4n-6	22:5n-3	22:5n-6	22:6n-3	unknown peak a	unknown peak b
LBFDGG (n=1)	4.6	0.2	0.0	2.6	26.6	34.2	0.8	4.6	2.0	0.3	0.8	0.7	0.1	0.1	2.7	1.6	2.8	9.7	0.4	0.0	0.3	2.2	0.2	1.9	0.2	0.3
LBFGKN (n=1)	4.7	0.2	0.0	3.0	25.0	34.6	0.8	4.3	2.2	0.3	0.8	0.7	0.2	0.1	3.1	1.7	2.5	10.4	0.3	0.0	0.4	2.4	0.1	2.0	0.2	0.2
LBFIHE (n=1)	4.8	0.1	0.0	3.0	23.7	34.1	0.9	3.6	4.0	0.4	1.0	0.9	0.2	0.1	3.1	1.3	3.3	10.3	0.4	0.0	0.3	1.9	0.1	1.8	0.2	0.3
LBFLDI (n=1)	6.4	0.1	0.0	2.1	28.5	28.4	0.9	5.9	1.2	0.2	0.7	0.7	0.3	0.2	3.0	1.9	1.7	10.8	0.3	0.0	0.4	3.3	0.2	1.7	0.4	0.4
LBFPNF (n=1)	5.4	0.2	0.0	2.3	26.9	28.9	1.3	5.1	3.0	0.4	0.8	0.7	0.1	0.0	3.3	1.6	2.8	11.0	0.4	0.0	0.2	3.2	0.1	1.7	0.3	0.2
LBFNSQ (n=1)	4.7	0.1	0.0	2.4	23.6	34.2	1.0	4.7	2.2	0.4	0.7	0.0	0.3	0.1	4.3	2.3	2.3	10.2	0.4	0.0	0.5	2.2	0.1	2.0	0.4	0.7
LBFDGL (n=1)	4.9	0.1	0.0	2.5	23.9	32.2	1.0	6.0	1.7	0.3	0.7	0.7	0.1	0.1	4.6	2.9	1.4	10.1	0.4	0.0	0.3	2.0	0.2	2.2	1.0	0.3
LBFIEF (n=1)	4.7	0.1	0.0	3.0	27.0	37.2	0.6	5.1	1.4	0.2	1.0	0.7	0.2	0.3	4.3	2.6	0.9	5.9	0.4	0.0	0.5	1.4	0.1	1.2	0.7	0.2
LBFBAV (n=1)	5.9	0.2	0.0	2.5	23.9	27.6	1.0	5.7	1.8	0.3	0.9	0.7	0.1	0.3	10.2	7.6	0.3	4.2	0.5	0.0	0.0	2.0	0.0	1.1	2.6	0.4
LBFPNC (n=1)	4.7	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
LBFGHQ (n=1)	5.4	0.2	0.0	2.3	28.7	31.5	0.5	9.0	1.4	0.6	0.9	0.8	0.1	0.1	2.6	2.1	0.6	6.5	0.4	0.0	0.3	2.8	0.2	1.6	0.9	0.4
LBFAZB (n=1)	5.1	0.1	0.0	3.1	26.6	30.1	1.6	4.7	2.5	0.4	0.8	0.6	0.0	0.1	5.4	2.9	1.6	8.7	0.4	0.0	0.4	2.2	0.1	1.5	0.9	0.3
LBFGKW (n=1)	4.4	0.2	0.0	2.0	26.5	31.5	1.2	6.8	1.9	0.3	0.6	0.7	0.1	0.1	6.7	4.1	0.5	5.9	0.3	0.0	0.4	2.0	0.2	1.2	2.0	0.3
LBFNRU (n=1)	5.0	0.2	0.0	2.5	27.2	26.6	0.9	8.0	1.4	0.3	0.8	0.7	0.1	0.2	6.0	4.6	0.4	7.0	0.4	0.0	0.4	3.0	0.1	1.8	1.9	0.3
LBFGIZ (n=1)	5.2	0.2	0.0	2.3	27.3	31.2	0.7	6.1	1.5	0.2	0.7	0.4	0.2	0.2	5.3	3.1	1.0	7.4	0.4	0.0	0.5	2.8	0.1	1.4	1.4	0.5

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LBFIGM (n=1)	4.9	0.1	0.0	2.5	28.9	29.6	1.6	5.8	1.8	0.3	0.8	0.8	0.1	0.1	5.0	2.5	1.5	7.9	0.4	0.0	0.4	2.2	0.1	1.5	0.9	0.4
LBFNRR (n=1)	4.8	0.1	0.0	2.8	27.3	26.9	1.9	6.5	1.9	0.3	0.9	0.8	0.1	0.1	5.4	3.7	0.8	8.9	0.4	0.0	0.3	2.5	0.1	1.6	1.1	0.5
LBFNTK (n=1)	4.9	0.2	0.0	2.4	29.0	27.5	1.1	6.8	1.4	0.2	0.7	0.7	0.2	0.1	5.9	3.6	1.0	7.3	0.4	0.0	0.6	3.0	0.1	1.1	1.5	0.4
LBFGJA (n=1)	5.2	0.2	0.0	3.0	27.1	33.5	2.1	3.4	5.2	0.6	0.8	0.7	0.1	0.1	4.2	1.6	1.5	5.4	0.3	0.0	0.7	1.6	0.2	1.2	1.0	0.2
LBFIFV (n=1)	4.8	0.1	0.0	3.4	21.6	34.4	0.8	3.5	2.8	0.3	0.9	0.7	0.2	0.1	6.8	2.9	2.2	7.6	0.4	0.0	0.6	1.8	0.3	2.6	1.0	0.2
LBFLER (n=1)	5.1	0.2	0.0	2.8	22.8	32.5	1.1	3.7	4.1	0.5	0.8	0.7	0.1	0.1	6.4	3.2	1.7	7.9	0.3	0.0	0.7	2.1	0.1	1.9	1.1	0.2
LBFLDL (n=1)	4.7	0.1	0.0	3.4	26.9	33.7	1.0	4.4	1.8	0.2	0.9	0.7	0.1	0.1	4.6	2.6	1.2	7.8	0.4	0.0	0.6	2.4	0.1	1.3	0.8	0.3
LBFNQW (n=1)	4.8	0.1	0.0	2.7	24.8	33.0	1.1	4.3	2.0	0.3	1.6	0.9	0.2	0.1	5.1	3.0	1.3	8.6	0.3	0.0	0.5	2.5	0.1	1.4	0.9	0.4
LBFBAP (n=1)	4.9	0.1	0.0	3.2	24.5	31.9	1.4	3.2	2.7	0.3	0.8	0.7	0.2	0.1	7.6	3.1	2.5	7.6	0.3	0.0	0.3	1.9	0.0	1.3	0.7	0.4
LBFDAU (n=1)	4.6	0.2	0.0	3.4	18.7	31.8	0.9	4.1	2.9	0.4	0.8	0.7	0.2	0.1	4.2	3.4	1.5	15.1	0.3	0.0	0.2	2.5	0.1	3.0	0.4	0.3
LBFPRA (n=1)	4.8	0.1	0.0	3.2	21.9	28.7	1.8	4.4	2.7	0.3	0.9	0.8	0.1	0.1	3.6	2.0	3.4	15.6	0.4	0.0	0.2	2.5	0.0	1.8	0.1	0.5
LBFIFU (n=1)	4.8	0.1	0.0	3.2	22.4	34.7	0.9	4.5	2.5	0.3	0.7	0.6	0.2	0.1	4.2	2.3	2.1	9.6	0.2	0.0	0.5	2.5	0.2	2.3	0.6	0.2
LBFDKD (n=1)	5.0	0.2	0.0	2.4	28.9	35.2	0.6	5.3	1.0	0.1	0.7	0.8	0.3	0.2	4.0	2.1	1.2	6.2	0.4	0.0	0.6	2.5	0.1	1.0	0.9	0.3
LBFDJG (n=1)	4.4	0.1	0.0	3.3	25.4	32.3	1.2	3.5	2.3	0.5	0.9	0.7	0.1	0.1	5.5	2.6	2.1	9.0	0.4	0.0	0.4	1.8	0.1	1.9	0.6	0.4
LBFLFK (n=1)	5.2	0.2	0.0	2.5	26.2	29.0	1.2	5.9	1.7	0.3	0.8	0.7	0.1	0.1	3.4	2.4	1.3	11.4	0.4	0.0	0.5	3.4	0.1	2.3	0.6	0.3
LBFLCG (n=1)	5.0	0.2	0.0	4.4	22.6	34.7	0.7	4.1	2.1	0.3	1.1	0.6	0.1	0.1	3.4	2.1	1.6	10.1	0.4	0.0	0.5	2.6	0.1	2.3	0.6	0.2
LBFPQM (n=1)	5.1	0.2	0.0	3.3	25.7	30.4	1.4	4.4	1.9	0.3	1.0	0.9	0.2	0.2	4.3	2.5	1.8	10.2	0.4	0.0	0.4	2.6	0.1	1.8	0.6	0.5
LBFDHG (n=1)	5.4	0.2	0.0	2.1	23.9	33.5	1.1	4.0	2.6	0.3	0.7	0.7	0.1	0.1	3.0	1.8	1.7	11.4	0.4	0.0	0.6	2.4	0.3	2.8	0.5	0.2
LBFDKA (n=1)	5.6	0.2	0.0	3.2	20.9	30.0	1.3	3.9	1.9	0.2	1.0	1.0	0.3	0.2	8.3	5.8	0.7	7.6	0.3	0.0	0.4	2.2	0.1	1.8	2.0	0.9
LBFIDT (n=1)	5.1	0.2	0.0	2.6	26.4	32.0	1.0	5.7	2.0	0.4	0.8	0.7	0.1	0.1	3.5	2.2	1.3	9.1	0.4	0.0	0.6	2.8	0.1	2.0	0.7	0.2

Table 140: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column; as defined in Table 131. In addition to those categories, the catergory "dc" was sub-divided into the category dc sl: all T1 plants where the average of all copy number assays listed in Table 135 was 3.51-4.49, and the zygocity analysis listed in Table 137 revelead a single locus insertion of both copies, and into the category dc dl: all T1 plants where the average of all copy number assays listed in Table 135 was 3.51-4.49, and the zygocity analysis listed in Table 137 revelead a double locus insertion of both copies. The number of T1 plants fullfilling these criteria are displayed in parentheses. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b

sc (n=172)	5.1 ± 0.9	0.2 ± 0.1	0 ± 0	2.8 ± 0.5	29.1 ± 3	33.8 ± 2.8	0.8 ± 0.2	4.8 ± 0.9	1.9 ± 0.7	0.3 ± 0.2	0.8 ± 0.1	0.8 ± 0.1	0.2 ± 0.2	0.1 ± 0.1	2.8 ± 0.3	1.5 ± 0.2	2.1 ± 0.4	7.9 ± 1.3	0.4 ± 0	0 ± 0	0.3 ± 0.1	1.9 ± 0.4	0.1 ± 0.1	1.4 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
dc (n=813)	4.9 ± 0.4	0.2 ± 0	0 ± 0	2.9 ± 0.5	29.1 ± 2.6	32 ± 3.1	1.1 ± 0.3	5.3 ± 1.3	1.9 ± 1	0.3 ± 0.1	0.9 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	5.9 ± 1.7	3.1 ± 1.1	0.9 ± 0.5	5.1 ± 2.1	0.4 ± 0	0 ± 0	0.4 ± 0.1	1.7 ± 0.5	0.1 ± 0.6	1 ± 0.4	1.4 ± 0.7	0.3 ± 0.1
dc sl (n=700)	4.9 ± 0.4	0.2 ± 0	0 ± 0	2.8 ± 0.4	29.4 ± 2.5	31.9 ± 3.2	1.1 ± 0.3	5.4 ± 1.4	1.9 ± 1.1	0.3 ± 0.1	0.8 ± 0.2	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	6 ± 1.7	3.2 ± 1.2	0.9 ± 0.6	4.8 ± 1.7	0.4 ± 0	0 ± 0	0.4 ± 0.2	1.7 ± 0.5	0.1 ± 0.6	0.9 ± 0.3	1.4 ± 0.7	0.3 ± 0.2
dc dl (n=64)	5 ± 0.5	0.2 ± 0	0 ± 0	3.2 ± 0.6	26.3 ± 2.5	32.2 ± 2.4	1.1 ± 0.2	4.5 ± 0.7	1.9 ± 0.4	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	4.3 ± 1.1	2.5 ± 0.5	1.4 ± 0.3	9 ± 2	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.4 ± 0.5	0.1 ± 0.1	1.7 ± 0.5	0.7 ± 0.3	0.3 ± 0.1

Table 141: Fatty acid profiles of T2 seeds harvested from T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column as defined in the description of Table 140. For each category, the fatty acid profile of the plant having the highest EPA+DHA levels was shown. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Category of T1 plants	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
sc (n=1)	6.4	0.1	0.0	3.0	23.7	34.1	0.9	3.6	4.0	0.4	1.0	0.9	0.2	0.1	3.1	1.3	3.3	10.3	0.4	0.0	0.3	1.9	0.1	1.8	0.2	0.3
dc (n=1)	4.6	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
dc sl (n=1)	4.8	0.1	0.0	2.3	19.8	35.1	0.6	3.9	2.7	0.3	0.6	0.7	0.2	0.1	4.3	1.8	4.6	11.4	0.3	0.0	0.6	3.9	0.1	1.1	0.4	0.3
dc dl (n=1)	4.6	0.3	0.0	2.7	20.4	31.3	0.8	3.9	2.3	0.3	0.7	0.5	0.2	0.1	3.9	2.5	2.4	13.7	0.3	0.0	0.3	2.6	0.2	2.9	0.4	0.2

Table 142: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBFDGG (n=50)	43.6 ± 2.7	9 ± 0	9 ± 0	9 ± 0	8.1 ± 1.6	3 ± 0	5 ± 0	5 ± 0	8.1 ± 1.6	4.5 ± 0.8	118.8 ± 6.1	4.4 ± 0.3	3.5 ± 0.9	36.1	30.2

LBFGKN (n=50)	43.3 ± 1.7	9 ± 0	9 ± 0	9 ± 0	8.1 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.1 ± 0.7	5.1 ± 0.8	112.4 ± 4.1	4.4 ± 0.3	3 ± 0.8	36.9	30.1
LBFIHE (n=34)	47.4 ± 5.1	9 ± 0	9 ± 0	9 ± 0	6.6 ± 1.6	3 ± 0	5 ± 0	5 ± 0	6.6 ± 1.6	5.6 ± 0.9	105.7 ± 6.3	4.2 ± 0.5	3.8 ± 0.6	36.0	29.4
LBFLDI (n=60)	35.8 ± 2.9	9 ± 0	7.5 ± 0.6	8.9 ± 0.7	8.2 ± 1.1	3 ± 0	4 ± 0.5	4 ± 0	8.4 ± 1.2	5.4 ± 0.9	94 ± 5.1	3.6 ± 0.8	4.8 ± 1.8	36.9	30.1
LBFPNF (n=52)	35.3 ± 2.2	9 ± 0	7.6 ± 0.6	8.8 ± 0.5	8.4 ± 0.6	3 ± 0	3.9 ± 0.3	4 ± 0	8.3 ± 1.2	5.3 ± 0.9	88.4 ± 7.9	3.6 ± 0.9	3.5 ± 0.7	39.1	28.3
LBFNSQ (n=51)	45.2 ± 7	9 ± 0	7.7 ± 0.9	6.8 ± 1.3	6.8 ± 1.2	3 ± 0	5 ± 0.2	4.1 ± 0.5	8.7 ± 0.6	5 ± 1	109.6 ± 10.9	3.4 ± 0.3	4.4 ± 1.8	37.5	28.7
LBFDGL (n=57)	43.9 ± 2.4	9 ± 0	9 ± 0	9 ± 0	7.7 ± 0.8	3 ± 0	5 ± 0	5 ± 0	7.7 ± 0.8	5.1 ± 0.8	109.4 ± 6.6	4.1 ± 0.4	2.8 ± 0.7	36.9	29.9
LBFIEF (n=6)	47.2 ± 5	9 ± 0	9 ± 0	9 ± 0	4 ± 2	3 ± 0	5 ± 0	5 ± 0	4 ± 2	6.2 ± 0.8	98.3 ± 4.1	3.4 ± 0.3	3.8 ± 1	38.9	29.9
LBFBAV (n=50)	46.6 ± 1.6	9 ± 0	7.4 ± 0.8	9 ± 0	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	9 ± 0	3.5 ± 0.5	125.1 ± 5.6	2.7 ± 0.3	3.9 ± 1	39.0	28.0
LBFPNC (n=32)	44.6 ± 4.2	9 ± 0	9 ± 0	9 ± 0	8.3 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.3 ± 0.7	5.5 ± 0.9	105.6 ± 5.6	4.1 ± 0.3	2.8 ± 0.5	39.4	28.3
LBFGHQ (n=46)	44.4 ± 4	9 ± 0	7.3 ± 1.9	9 ± 0	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	9 ± 0.1	3.9 ± 0.7	124.5 ± 6.2	2.6 ± 0.3	3.7 ± 0.9	38.3	27.8
LBFAZB (n=49)	47.6 ± 3.2	9 ± 0	9 ± 0	9 ± 0	7.9 ± 0.8	3 ± 0	5 ± 0	5 ± 0	7.9 ± 0.8	5.1 ± 0.8	112.3 ± 4.9	3.9 ± 0.4	2.6 ± 0.6	38.2	29.4
LBFGKW (n=72)	46.7 ± 1.9	9 ± 0	7.3 ± 0.5	9 ± 0	8.9 ± 0.3	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.3	4.1 ± 0.6	125.2 ± 6.2	2.6 ± 0.3	3.7 ± 0.8	38.8	27.9
LBFNRU (n=58)	41.4 ± 1.7	9 ± 0	7.6 ± 0.6	8.9 ± 0.7	8.2 ± 0.7	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.8	5.6 ± 0.8	117 ± 5	2.6 ± 0.3	3.6 ± 1.3	39.5	27.6
LBFGIZ (n=43)	41.5 ± 2.8	9 ± 0	7.6 ± 0.6	9 ± 0	8.3 ± 0.5	3 ± 0	5 ± 0	4 ± 0	8.9 ± 0.3	5.3 ± 0.9	113.5 ± 5.9	2.4 ± 0.2	3.9 ± 0.7	38.4	28.6
LBFIGM (n=56)	36.1 ± 1.3	9 ± 0	6.7 ± 0.8	9 ± 0.3	8.8 ± 0.4	3 ± 0	2.7 ± 0.7	4 ± 0	8.8 ± 0.5	3.9 ± 0.7	107.6 ± 9	2.7 ± 0.3	3.4 ± 0.8	39.1	28.6
LBFNRR (n=61)	41.3 ± 2.2	9 ± 0	7.8 ± 0.6	9 ± 0	8 ± 0.6	3 ± 0	5 ± 0	4 ± 0	9 ± 0	5.3 ± 0.6	116.5 ± 6.3	2.9 ± 0.3	3.2 ± 0.9	40.6	27.1
LBFNTK (n=69)	34.5 ± 2.8	8 ± 0	6.6 ± 1.1	9 ± 0	8.7 ± 0.5	3 ± 0	2.4 ± 0.6	4 ± 0	8.8 ± 0.7	3.7 ± 0.6	93.4 ± 8.2	2.8 ± 0.3	3.8 ± 0.8	39.8	28.3
LBFGJA (n=42)	45.1 ± 1	9 ± 0	9 ± 0	9 ± 0	7.6 ± 0.5	3 ± 0	5 ± 0	5 ± 0	7.6 ± 0.5	4.3 ± 1.1	113.3 ± 2.4	4.9 ± 0.2	2.4 ± 0.8	32.9	25.6
LBFIFV (n=58)	42.4 ± 0.6	9 ± 0	9 ± 0	9 ± 0	8.8 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.8 ± 0.6	4.5 ± 0.8	113.1 ± 2.4	4.2 ± 0.2	2.6 ± 0.6	37.3	29.4
LBFLER (n=52)	45.1 ± 3.5	9 ± 0	9 ± 0	9 ± 0	8.5 ± 0.7	3 ± 0	5 ± 0	5 ± 0	8.5 ± 0.7	5.6 ± 1	108.3 ± 6.8	3.9 ± 0.3	2.6 ± 0.7	40.8	27.7
LBFLDL (n=44)	44.7 ± 3.7	9 ± 0	9 ± 0	9 ± 0	8.5 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.5 ± 0.6	5.1 ± 0.8	109.3 ± 5.3	4 ± 0.2	2.6 ± 0.8	38.6	29.2
LBFNQW (n=51)	42.8 ± 1.9	9 ± 0	9 ± 0	9 ± 0	8.7 ± 0.5	3 ± 0	5 ± 0	5 ± 0	8.7 ± 0.5	5.7 ± 0.9	105.5 ± 6.3	4.1 ± 0.3	3.1 ± 0.5	38.4	28.9
LBFBAP (n=19)	43.3 ± 1.8	9 ± 0	9 ± 0	9 ± 0	8.9 ± 0.2	3 ± 0	5 ± 0	5 ± 0	8.9 ± 0.2	4.6 ± 0.8	117.4 ± 6.1	4 ± 0.5	2.4 ± 0.5	38.7	28.8
LBFDAU (n=10)	43.6 ± 1.7	9 ± 0	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	8.5 ± 1.6	4.4 ± 0.8	110 ± 5.8	4.5 ± 0.5	2.6 ± 0.5	34.5	30.2
LBFPRA (n=16)	47.8 ± 2.5	9 ± 0	7.9 ± 0.8	7.8 ± 0.4	7.4 ± 0.8	3 ± 0	5.2 ± 0.4	4 ± 0	9 ± 0	5.6 ± 0.8	123.4 ± 4	4.2 ± 0.6	2.3 ± 0.8	38.1	28.2
LBFIFU (n=11)	46.8 ± 6.4	9 ± 0	8.7 ± 0.9	8.7 ± 0.9	7.3 ± 1.1	3 ± 0	5 ± 0	5 ± 0	7.3 ± 1.1	5.6 ± 1.1	103.6 ± 8.1	3.8 ± 0.5	3.5 ± 0.9	37.4	28.7
LBFDKD (n=2)	43 ± 4.2	9 ± 0	9 ± 0	9 ± 0	7.5 ± 0.7	3 ± 0	5 ± 0	5 ± 0	7.5 ± 0.7	5 ± 1.4	107.5 ± 10.6	4.3 ± 0.8	4 ± 1.4	36.6	31.2
LBFDJG (n=12)	45.1 ± 1	9 ± 0	9 ± 0	9 ± 0	8.8 ± 0.6	3 ± 0	5 ± 0	5 ± 0	8.8 ± 0.6	4.3 ± 1	104.2 ± 29.8	3.8 ± 0.2	2.8 ± 0.5	37.7	29.8
LBFLFK (n=15)	42.5 ± 5.5	9 ± 0	7.9 ± 1.2	8.7 ± 1.3	8.1 ± 1.3	3 ± 0	4.9 ± 0.5	4 ± 0	8.9 ± 0.4	5.1 ± 1.2	113.7 ± 9	4.1 ± 1	3.7 ± 0.6	39.3	27.2
LBFLCG (n=15)	42.5 ± 0.6	9 ± 0	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	8.7 ± 1	4.5 ± 0.8	113.7 ± 2.3	4.3 ± 0.5	2.7 ± 0.8	37.7	29.1
LBFPQM (n=12)	42.7 ± 2.5	9 ± 0	9 ± 0	9 ± 0	8.7 ± 0.5	3 ± 0	5 ± 0	5 ± 0	8.7 ± 0.5	5.5 ± 1	110 ± 6	4.2 ± 0.5	2.8 ± 0.4	39.2	27.7
LBFDHG (n=5)	44.8 ± 3	9 ± 0	9 ± 0	9 ± 0	6.4 ± 0.9	3 ± 0	5 ± 0	5 ± 0	6.4 ± 0.9	4.2 ± 0.8	115 ± 0	4.2 ± 0.2	5 ± 0	33.5	28.0
LBFDKA (n=5)	48.6 ± 1.7	9 ± 0	9 ± 0	9 ± 0	9 ± 0	3 ± 0	5 ± 0	5 ± 0	9 ± 0	5.6 ± 0.9	108 ± 4.5	3.8 ± 0.2	2.4 ± 0.5	40.0	26.6

LBFIDT (n=7)	46.3 ± 1.3	9 ± 0	7.7 ± 0.5	9 ± 0	8 ± 0	3 ± 0	5 ± 0	4 ± 0	9 ± 0	4.1 ± 0.7	125.7 ± 6.7	2.5 ± 0.2	3.7 ± 0.5	38.1	28.0

Table 143: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. Plants of all events combined have been grouped into the categories indicated in the first column as defined in the description of Table 140. The number of T1 plants fullfilling these criteria are displayed in parentheses. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoI: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Category of T1 plants	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	oil	protein
sc (n=177)	41.5 ± 5.2	9 ± 0	8.6 ± 0.7	9 ± 0.4	7.9 ± 1.5	3 ± 0	4.8 ± 0.5	4.8 ± 0.4	8 ± 1.5	5.1 ± 1	109.1 ± 10.6	3.9 ± 0.6	3.8 ± 1.4	37 ± 1.4	29.5 ± 0.9
dc (n=781)	43.4 ± 4.4	8.9 ± 0.3	8.1 ± 1.1	9 ± 0.3	8.4 ± 0.8	3 ± 0	4.6 ± 0.9	4.5 ± 0.5	8.6 ± 0.8	4.7 ± 1.1	112.2 ± 11	3.3 ± 0.8	3.2 ± 1	38 ± 2.1	28.5 ± 1.2
dc sl (n=677)	43.1 ± 4.4	8.9 ± 0.3	8 ± 1.1	9 ± 0.2	8.5 ± 0.8	3 ± 0	4.6 ± 1	4.4 ± 0.5	8.7 ± 0.8	4.7 ± 1.1	112.3 ± 11	3.3 ± 0.8	3.2 ± 1	38.4 ± 1.8	28.3 ± 1.1
dc dl (n=55)	43.3 ± 3.3	9 ± 0	8.7 ± 0.8	8.9 ± 0.6	8.4 ± 1	3 ± 0	5 ± 0.3	4.8 ± 0.4	8.5 ± 1.1	4.8 ± 1.1	110.8 ± 14.3	3.5 ± 0.7	3.2 ± 0.9	36.6 ± 2.7	28.9 ± 1.7

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Fatty acid profiles, copy number measurements, and phenotypic observations of T1 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in field trials in USDA growth zone 11 during winter.

Certain events that had higher levels of EPA and DHA were tested in the field and examined for fatty acid profile, aerial phenotype (if any) and copy number in the T1 generation. A variety of constructs were examined including those with partial double copy insertions, single copy insertions and double copy insertions being represented (see Table 144). Table 145 indicates that LBFDAU had an EPA content of ca. 13% and a DHA content of ca. 3% of the total seed fatty acid content, and a maximum content for DHA of 3.6% and EPA of 17% of total seed fatty acids (Table 146). Measurements of single seeds from LBFDAU had as much as 26% EPA and 4.6% DHA, see Table 147. Overall the field performance of LBFDAU matched or exceeded that of the greenhouse.

Table 144: Copy number measurement of T1 plants cultivated in field, corresponding to USDA growth zone 11, during the winter for field trials of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where measured per event. The T1 plants underwent a selection from ~80 segregating T1 seedlings, using zygocity analysis similar to the selection performed in the greenhouse (which was illustrated in Table 137), keeping only plants that are homozygous for the desired number of loci. A copy number of ~2 therefore was indicative for one homozygous copy, a copy number of ~4 indicative for two homozygous copies (located either at on or at two different loci) and so forth. Odd results of 3, 5, 7, 9 etc indicate that at least some of the selected T1 plants carry at least one heterozygous locus. Homozygocity was indicated if the average result of the selected T1 plants was about two fold higher than the the result oberved in the T0 generation (indicated in parentheses). For some events this was not the case because during selection of T1 plants, undesired loci have been segregated out while retaining only desired loci in a homozygous state.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.														
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	j-p-BnSETL-v1_c-o3Des(Pir_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	Conclusion from individual assays: number of T-DNA copies inserted into the genome
LBFDGG (n=5)	2 (T0: 1.1)										1.9 (T0: 1)			1.9 (T0: 1)	single copy
LBFGKN (n=12)	2.1 (T0: 1)										2 (T0: 1)			1.9 (T0: 1.1)	single copy
LBFHE (n=8)	2.1 (T0: 1)										1.9 (T0: 1.2)			1.9 (T0: 1.1)	single copy
LBFLDI (n=13)	2 (T0: 1)										1.9 (T0: 1)			1.8 (T0: 1)	single copy
LBFPNF (n=9)	2 (T0: 1.1)										3.7 (T0: 1.9)			3.3 (T0: 1.9)	partial double copy
LBFDAU (n=4)	2 (T0: 1.1)										3.8 (T0: 2.4)			3.6 (T0: 1.9)	partial double copy
LBFPRA (n=5)	3.2 (T0: 2)										4.9 (T0: 3.1)			4 (T0: 2.8)	partial double copy
LBFLFK (n=4)	3.9 (T0: 2)										3.4 (T0: 2.4)			3.4 (T0: 2)	partial double copy
LBFLCG (n=3)	4.3 (T0: 1.9)										2.9 (T0: 2.5)			3.4 (T0: 2)	partial double copy
LBFPQM (n=1)	3.7 (T0: 2)										(T0: 2)			3.9 (T0: 1.8)	partial double copy

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Table 145: Fatty acid profiles of T2 seeds harvested from T1 cultivated in the field, corresponding to USDA growth zone 11, during winter of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T2 seed batches that were measured per event. Per seed batch a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDGG (n=5)	4.4 ± 0.2	0.2 ± 0	0.1 ± 0	2.4 ± 0.1	32.3 ± 1.9	34.4 ± 1.6	0.7 ± 0	5.3 ± 0.6	1.5 ± 0.1	0.4 ± 0.1	0.6 ± 0	0.7 ± 0	0.1 ± 0	0 ± 0	2 ± 0.2	1.1 ± 0.2	2.7 ± 0.3	6.9 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	1.9 ± 0.2	0.1 ± 0	1.4 ± 0.3	0 ± 0	0.1 ± 0
LBFGKN (n=12)	4.8 ± 0.3	0.2 ± 0	0.1 ± 0	2.6 ± 0.3	27.8 ± 1.9	36 ± 1.3	0.5 ± 0.1	4.8 ± 0.7	1.5 ± 0.2	0.4 ± 0.1	0.7 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0	2.3 ± 0.2	1.1 ± 0.2	3.3 ± 0.4	8 ± 0.8	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	1.9 ± 0.3	0.1 ± 0	1.8 ± 0.4	0.1 ± 0	0.2 ± 0
LBFIHE (n=8)	4.9 ± 0.8	0.3 ± 0.1	0.1 ± 0	2.4 ± 0.4	30.8 ± 3.1	36.1 ± 1.2	0.5 ± 0.1	5.4 ± 1.3	1.5 ± 0.5	0.3 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.1 ± 0	1.9 ± 0.5	0.9 ± 0.1	2.8 ± 0.7	6.4 ± 1.1	0.3 ± 0	0 ± 0	0.5 ± 0.1	1.7 ± 0.3	0.1 ± 0	1.3 ± 0.2	0 ± 0.1	0.1 ± 0
LBFLDI (n=13)	4.5 ± 1.1	0.2 ± 0.1	0.1 ± 0	2.3 ± 0.6	30 ± 5.9	35.4 ± 6.1	0.5 ± 0.1	4.1 ± 0.8	1.3 ± 0.3	0.3 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.2 ± 0.1	0.1 ± 0	1.9 ± 0.5	0.8 ± 0.2	2.9 ± 0.8	6.4 ± 1.6	0.3 ± 0.1	0 ± 0	0.4 ± 0.1	1.6 ± 0.3	0.1 ± 0.1	1.3 ± 0.4	0.1 ± 0	0.1 ± 0
LBFPNF (n=9)	4.7 ± 0.2	0.2 ± 0	0.1 ± 0	2.4 ± 0.3	29.2 ± 1.8	31.7 ± 1	1.1 ± 0.1	4 ± 0.6	2.6 ± 1.4	0.6 ± 0.1	0.7 ± 0	0.7 ± 0	0.1 ± 0	0 ± 0	3.3 ± 0.4	1.2 ± 0.1	4.2 ± 0.5	8.4 ± 1.1	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.4	0.1 ± 0	1.5 ± 0.3	0.1 ± 0.1	0.1 ± 0
LBFDAU (n=4)	4.6 ± 0.2	0.2 ± 0	0.1 ± 0	2.3 ± 0.3	24.3 ± 3.7	31.2 ± 2.5	0.8 ± 0.1	5.2 ± 0.6	2.2 ± 0.5	0.4 ± 0.1	0.6 ± 0	0.7 ± 0.1	0.1 ± 0	0.1 ± 0	2.5 ± 0.4	1.7 ± 0.5	2.9 ± 0.4	13.4 ± 3.4	0.3 ± 0	0 ± 0	0.3 ± 0	2.7 ± 0.7	0.1 ± 0	2.7 ± 0.7	0.1 ± 0.1	0.2 ± 0
LBFPRA (n=5)	4.8 ± 0.2	0.2 ± 0	0.1 ± 0	2.5 ± 0.4	25.1 ± 0.9	34.5 ± 2.4	0.9 ± 0.2	4.4 ± 0.7	2.2 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.6 ± 0.6	1.3 ± 0.3	3.6 ± 0.6	10.7 ± 1.9	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.3 ± 0.3	0.1 ± 0	1.7 ± 0.2	0.1 ± 0.1	0.2 ± 0.1
LBFLFK (n=4)	5.1 ± 0.8	0.2 ± 0.1	0.1 ± 0	2.1 ± 0.4	27 ± 4.3	32.3 ± 0.5	0.8 ± 0.1	5.4 ± 0.8	1.9 ± 0.5	0.4 ± 0	0.6 ± 0.1	0.7 ± 0.1	0.2 ± 0	0.1 ± 0	3.1 ± 0.6	1.7 ± 0.3	2.7 ± 0.5	9.5 ± 1.8	0.3 ± 0	0 ± 0	0.5 ± 0.2	2.9 ± 0.4	0.2 ± 0.1	2.1 ± 0.5	0.3 ± 0.1	0.2 ± 0
LBFLCG (n=3)	4.8 ± 0.2	0.2 ± 0	0.1 ± 0	3 ± 0.3	26.4 ± 1.3	33.3 ± 1	0.8 ± 0	4.7 ± 0.7	2 ± 0.2	0.5 ± 0.1	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.3 ± 0.1	1.2 ± 0.1	2.9 ± 0.2	9.9 ± 0.9	0.3 ± 0	0 ± 0	0.7 ± 0.1	2.6 ± 0.3	0.2 ± 0	2.3 ± 0.5	0.2 ± 0	0.2 ± 0
LBFPQM (n=1)	5.0	0.2	0.1	2.7	26.1	30.8	1.0	4.3	2.3	0.5	0.7	0.6	0.1	0.1	3.4	1.8	2.8	10.8	0.3	0.0	0.5	2.5	0.3	2.5	0.4	0.2

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Table 146: Fatty acid profiles of one T2 seed batch per event harvested from T1 plants cultivated in the field, corresponding to USDA growth zone 11, during winter of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T2 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4n-3	20:4 n-6	20:5n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDGG (n=1)	4.5	0.21	0.1	2.4	29.86	33.45	0.71	5.74	1.69	0.45	0.62	0.74	0.12	0.05	2.25	1.38	2.83	8.02	0.29	0	0.35	2.21	0.1	1.79	0	0.13
LBFGKN (n=1)	4.5	0.21	0.09	2.28	26.68	34.29	0.65	4.99	1.73	0.42	0.61	0.69	0.23	0.1	2.43	1.22	3.34	9.74	0.31	0	0.48	2.27	0.17	2.31	0	0.23
LBFIHE (n=1)	4.8	0.22	0.09	2.95	26.2	38.09	0.52	3.19	2.12	0.22	0.78	0.69	0.15	0	2.73	1.03	3.67	8.16	0.35	0	0.59	1.72	0.12	1.55	0	0.09
LBFLDI (n=1)	4.8	0.24	0.09	2.51	27.6	35.76	0.54	4.15	1.66	0.26	0.7	0.69	0.29	0.1	2.63	1.16	3.5	8.32	0.31	0	0.47	1.89	0.19	1.85	0.15	0.21
LBFPNF (n=1)	4.9	0.27	0.13	2.11	26.29	31.14	0.91	5.04	1.72	0.68	0.67	0.7	0.11	0.04	2.8	1.34	4.26	10.24	0.36	0	0.54	3.24	0.14	2.09	0.15	0.12
LBFDAU (n=1)	4.8	0.23	0.14	1.94	20.37	28.27	0.98	5.23	2.78	0.5	0.63	0.62	0.13	0.13	3.08	2.3	2.51	17.57	0.36	0	0.29	3.16	0.09	3.62	0	0.25
LBFPRA (n=1)	4.7	0.16	0.14	1.92	25.56	32.27	1.21	4.87	2.09	0.44	0.51	0.66	0.13	0.07	2.34	1.29	3.67	12.46	0.28	0	0.44	2.58	0.08	1.8	0.08	0.26
LBFLFK (n=1)	4.9	0.21	0.12	2.34	22.43	32.9	0.9	4.55	2.5	0.42	0.66	0.62	0.13	0.07	3.86	2.18	2.37	11.06	0.3	0	0.76	3.2	0.24	2.66	0.46	0.21
LBFLCG (n=1)	5.0	0.27	0.12	2.65	24.88	32.2	0.74	5.16	2.3	0.46	0.69	0.65	0.14	0.09	2.24	1.36	2.85	10.87	0.31	0	0.69	2.92	0.22	2.82	0.21	0.15
LBFPQM (n=1)	5.0	0.22	0.11	2.67	26.07	30.83	1.02	4.34	2.31	0.45	0.69	0.63	0.12	0.07	3.4	1.75	2.78	10.78	0.3	0	0.54	2.53	0.27	2.5	0.4	0.23

Table 147: Fatty acid profiles of 95 single seeds of the one seedbatch of event LBFDAU shown in Table 146 having highest EPA+DHA levels .

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1n-9	18:2n-6	18:2n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4n-3	20:4 n-6	20:5n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU seed 1	4.2	0.2	0.2	2.1	11.3	26.5	1.2	3.8	4.3	0.6	0.7	0.5	0.1	0.1	3.3	3.0	1.9	26.2	0.4	0.0	0.6	3.7	0.2	4.9	0.0	0.2
LBFDAU seed 2	5.5	0.3	0.1	2.5	14.3	25.0	0.8	4.5	2.5	0.5	0.7	0.5	0.2	0.1	3.0	2.5	2.5	23.6	0.5	0.0	0.5	3.8	0.3	5.8	0.0	0.2
LBFDAU seed 3	5.1	0.3	0.1	2.0	16.0	25.5	1.2	4.4	3.4	0.6	0.7	0.5	0.1	0.1	3.4	2.5	2.5	23.1	0.3	0.0	0.6	3.3	0.1	4.2	0.0	0.2
LBFDAU seed 4	6.7	0.4	0.2	3.8	15.8	23.5	1.5	4.9	3.3	0.9	1.2	0.5	0.0	0.0	1.7	1.3	1.9	21.2	0.7	0.0	0.8	3.5	0.4	5.8	0.0	0.0
LBFDAU seed 5	5.4	0.4	0.2	1.5	16.1	24.4	1.0	5.4	3.4	0.8	0.6	0.5	0.1	0.1	2.9	2.1	3.5	22.0	0.3	0.0	0.5	3.7	0.2	4.8	0.0	0.2
LBFDAU seed 6	5.5	0.3	0.1	1.9	16.6	25.6	0.9	5.3	3.3	0.7	0.7	0.6	0.1	0.1	2.9	2.3	2.4	21.8	0.3	0.0	0.4	3.4	0.2	4.4	0.0	0.2
LBFDAU seed 7	5.0	0.4	0.2	2.4	16.3	25.3	1.1	4.6	3.5	0.6	0.7	0.5	0.1	0.0	3.6	3.1	2.0	21.9	0.5	0.0	0.5	3.5	0.0	4.3	0.0	0.1
LBFDAU seed 8	5.3	0.3	0.1	2.3	17.4	26.0	1.2	4.1	3.7	0.7	0.7	0.5	0.1	0.1	2.6	2.0	2.7	21.7	0.4	0.0	0.4	3.1	0.2	4.0	0.0	0.2
LBFDAU seed 9	5.3	0.2	0.2	2.4	14.3	27.8	1.0	4.0	4.2	0.6	0.7	0.5	0.1	0.1	3.6	2.9	2.3	21.1	0.4	0.0	0.5	2.8	0.3	4.4	0.0	0.1

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LBFDAU seed 10	6.7	0.5	0.2	2.8	15.8	23.3	0.8	5.7	2.9	0.8	0.9	0.5	0.0	0.0	3.1	2.4	2.2	19.8	0.8	0.0	0.6	4.7	0.1	5.4	0.0	0.0
LBFDAU seed 11	5.9	0.6	0.2	3.4	19.3	22.2	1.5	4.0	3.1	0.7	0.9	0.6	0.0	0.0	3.0	2.1	2.0	20.8	0.8	0.0	0.2	4.6	0.0	4.2	0.0	0.0
LBFDAU seed 12	5.2	0.4	0.2	2.2	16.1	25.2	0.8	5.5	3.3	0.7	0.8	0.5	0.1	0.1	3.7	2.9	2.4	20.1	0.4	0.0	0.4	4.2	0.2	4.7	0.0	0.2
LBFDAU seed 13	4.3	0.2	0.1	2.0	17.5	27.7	1.2	4.9	2.9	0.5	0.6	0.6	0.1	0.1	2.9	2.3	2.6	20.7	0.3	0.0	0.4	3.6	0.2	4.1	0.0	0.2
LBFDAU seed 14	4.2	0.3	0.1	2.0	16.4	27.9	0.9	5.1	2.9	0.5	0.6	0.5	0.1	0.1	3.5	3.2	2.0	20.5	0.3	0.0	0.4	3.7	0.1	4.3	0.0	0.2
LBFDAU seed 15	4.5	0.4	0.1	1.5	17.0	25.7	1.1	5.3	2.9	0.6	0.5	0.6	0.1	0.1	3.4	2.4	2.9	19.7	0.3	0.0	0.5	4.8	0.3	5.1	0.0	0.3
LBFDAU seed 16	4.7	0.6	0.2	2.0	17.2	25.9	1.1	5.9	3.0	0.7	0.7	0.5	0.1	0.0	3.2	2.6	2.3	20.1	0.6	0.0	0.7	3.4	0.1	4.3	0.0	0.2
LBFDAU seed 17	4.5	0.3	0.1	2.3	18.4	27.0	1.5	4.6	3.4	0.7	0.7	0.6	0.1	0.0	2.7	1.9	2.8	20.8	0.5	0.0	0.6	2.8	0.1	3.5	0.0	0.2
LBFDAU seed 18	5.1	0.2	0.2	2.9	16.1	27.1	1.5	4.7	4.2	0.7	0.9	0.7	0.0	0.0	2.3	2.3	2.1	19.5	0.7	0.0	0.4	3.4	0.0	4.8	0.0	0.2
LBFDAU seed 19	4.5	0.3	0.1	1.7	18.7	27.0	1.4	4.5	2.9	0.4	0.5	0.6	0.1	0.1	3.1	2.1	2.9	19.8	0.2	0.0	0.5	3.7	0.2	4.3	0.0	0.4
LBFDAU seed 20	5.3	0.2	0.1	2.6	17.9	26.4	1.1	4.4	2.9	0.5	0.7	0.6	0.1	0.1	3.4	2.7	2.8	20.8	0.3	0.0	0.4	3.0	0.1	3.3	0.0	0.2
LBFDAU seed 21	5.2	0.3	0.2	2.1	16.3	27.8	0.7	5.1	3.5	0.7	0.7	0.6	0.2	0.1	3.1	2.4	2.7	19.7	0.4	0.0	0.5	3.6	0.2	4.0	0.0	0.1
LBFDAU seed 22	4.8	0.2	0.1	2.4	17.7	28.9	0.9	4.9	3.1	0.5	0.7	0.6	0.1	0.1	2.4	2.1	2.7	19.9	0.3	0.0	0.4	3.0	0.1	3.8	0.0	0.2
LBFDAU seed 23	4.5	0.2	0.1	1.6	19.4	26.9	1.3	5.6	2.8	0.5	0.5	0.6	0.1	0.1	3.0	2.3	3.1	19.1	0.2	0.0	0.4	3.7	0.1	3.7	0.0	0.2
LBFDAU seed 24	6.6	0.6	0.2	3.4	18.4	23.0	1.0	3.9	4.6	1.1	1.1	0.7	0.0	0.0	3.6	2.0	1.6	17.9	0.7	0.0	0.9	3.7	0.0	4.8	0.0	0.0
LBFDAU seed 25	5.2	0.3	0.2	2.1	16.0	26.6	0.9	4.9	3.7	0.8	0.7	0.6	0.0	0.0	3.9	2.6	2.8	18.0	0.6	0.0	0.7	4.6	0.3	4.4	0.0	0.1
LBFDAU seed 26	5.5	0.6	0.2	2.6	19.9	23.4	2.1	3.8	3.0	0.6	0.8	0.6	0.0	0.0	4.0	2.0	2.8	18.5	0.6	0.0	1.0	3.8	0.2	3.9	0.0	0.2
LBFDAU seed 27	4.5	0.3	0.1	1.7	19.5	26.6	1.5	5.2	2.3	0.5	0.5	0.6	0.1	0.1	3.6	2.5	2.6	18.6	0.3	0.0	0.5	4.1	0.2	3.8	0.0	0.4

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LBFDAU seed 28	5.1	0.3	0.2	2.5	17.2	27.3	0.8	4.6	2.9	0.5	0.9	0.5	0.1	0.0	3.7	2.3	3.6	18.6	0.5	0.0	0.5	3.7	0.2	3.8	0.0	0.2
LBFDAU seed 29	5.0	0.3	0.2	3.2	20.0	28.0	1.3	4.7	2.9	0.5	0.9	0.6	0.1	0.0	2.2	1.5	2.3	18.5	0.4	0.0	0.5	2.8	0.2	3.6	0.0	0.2
LBFDAU seed 30	4.7	0.2	0.1	2.4	18.6	29.3	1.6	4.4	3.2	0.5	0.7	0.6	0.1	0.1	2.9	2.1	2.5	18.6	0.3	0.0	0.7	2.7	0.2	3.2	0.0	0.2
LBFDAU seed 31	4.9	0.3	0.1	2.5	18.8	28.0	1.5	4.1	3.0	0.4	0.8	0.6	0.1	0.1	3.9	2.8	1.8	18.2	0.4	0.0	0.6	3.1	0.2	3.6	0.0	0.3
LBFDAU seed 32	4.8	0.3	0.1	2.2	19.6	28.3	1.0	5.5	2.6	0.5	0.6	0.6	0.1	0.1	3.1	2.5	2.4	18.3	0.3	0.0	0.4	3.1	0.1	3.4	0.0	0.2
LBFDAU seed 33	4.6	0.3	0.1	2.3	19.6	28.4	1.3	5.2	3.1	0.6	0.8	0.6	0.1	0.0	2.7	2.0	2.4	18.2	0.4	0.0	0.5	3.1	0.1	3.5	0.0	0.2
LBFDAU seed 34	4.9	0.3	0.1	3.4	19.2	25.9	1.8	4.0	3.1	0.5	1.0	0.7	0.1	0.0	3.9	2.7	2.1	17.9	0.5	0.0	0.6	3.0	0.2	3.7	0.0	0.3
LBFDAU seed 35	4.6	0.3	0.2	2.0	19.1	28.5	1.1	5.7	3.0	0.6	0.6	0.6	0.1	0.1	3.0	2.3	2.6	17.9	0.3	0.0	0.4	3.3	0.1	3.6	0.0	0.2
LBFDAU seed 36	4.2	0.2	0.1	2.1	18.1	30.5	1.0	4.9	2.9	0.4	0.6	0.6	0.1	0.1	3.5	3.0	2.1	17.6	0.3	0.0	0.4	3.2	0.1	3.8	0.0	0.2
LBFDAU seed 37	4.9	0.2	0.1	2.0	19.0	29.0	1.1	5.0	3.0	0.5	0.6	0.6	0.1	0.1	3.1	2.2	2.9	17.8	0.3	0.0	0.4	3.0	0.2	3.5	0.0	0.2
LBFDAU seed 38	4.4	0.3	0.1	1.9	19.7	29.9	1.2	4.5	2.6	0.4	0.6	0.5	0.1	0.1	3.0	2.0	2.6	17.8	0.3	0.0	0.5	3.4	0.2	3.5	0.0	0.2
LBFDAU seed 39	4.8	0.2	0.1	2.2	19.2	28.6	1.0	5.0	2.4	0.4	0.6	0.6	0.1	0.1	4.0	3.2	1.9	17.8	0.3	0.0	0.5	3.1	0.1	3.4	0.0	0.3
LBFDAU seed 40	4.3	0.3	0.1	1.8	20.2	28.8	1.4	4.8	2.6	0.4	0.6	0.6	0.1	0.1	3.2	2.2	2.5	17.7	0.3	0.0	0.5	3.6	0.2	3.5	0.0	0.3
LBFDAU seed 41	5.8	0.4	0.2	3.0	19.3	26.9	0.9	4.6	3.6	0.7	1.0	0.6	0.0	0.0	3.1	1.8	2.1	17.7	0.7	0.0	1.0	3.1	0.1	3.5	0.0	0.0
LBFDAU seed 42	4.6	0.3	0.1	2.3	21.3	27.8	1.2	5.2	2.8	0.6	0.6	0.6	0.1	0.1	2.7	2.1	2.3	18.0	0.3	0.0	0.7	2.9	0.1	3.2	0.0	0.2
LBFDAU seed 43	5.3	0.3	0.2	2.0	18.1	29.2	1.0	4.2	3.1	0.5	0.7	0.6	0.1	0.1	3.4	2.1	3.7	17.6	0.3	0.0	0.5	3.5	0.2	3.4	0.0	0.2
LBFDAU seed 44	4.7	0.3	0.2	1.9	19.3	29.3	1.0	5.7	2.7	0.5	0.7	0.7	0.1	0.1	2.7	2.1	2.6	17.3	0.3	0.0	0.5	3.6	0.2	3.6	0.0	0.2
LBFDAU seed 45	4.3	0.2	0.1	2.2	19.4	29.8	1.1	5.0	2.5	0.4	0.7	0.6	0.1	0.1	3.3	2.6	2.4	17.7	0.3	0.0	0.5	3.2	0.2	3.2	0.0	0.2

LBFDAU seed 46	4.5	0.2	0.2	2.4	18.8	29.0	1.1	5.0	3.7	0.6	0.7	0.6	0.1	0.1	3.1	2.2	2.8	17.1	0.3	0.0	0.5	3.1	0.2	3.5	0.0	0.2
LBFDAU seed 47	4.0	0.2	0.1	1.8	19.2	30.1	1.3	4.9	2.9	0.4	0.6	0.6	0.1	0.1	3.5	2.5	2.6	17.0	0.3	0.0	0.5	3.5	0.2	3.5	0.0	0.3
LBFDAU seed 48	4.3	0.2	0.1	2.0	20.0	30.1	1.3	4.5	2.7	0.4	0.6	0.5	0.1	0.1	3.5	2.3	2.7	16.9	0.3	0.0	0.4	3.1	0.2	3.5	0.0	0.3
LBFDAU seed 49	4.4	0.2	0.1	2.5	18.3	30.1	1.6	3.7	3.2	0.5	0.8	0.6	0.1	0.1	4.3	3.3	2.0	17.2	0.4	0.0	0.4	2.5	0.2	3.2	0.0	0.3
LBFDAU seed 50	4.3	0.3	0.1	1.9	21.5	29.2	1.3	5.2	2.5	0.5	0.6	0.6	0.1	0.1	2.8	2.1	2.3	17.0	0.3	0.0	0.4	3.2	0.2	3.3	0.0	0.2
LBFDAU seed 70	5.3	0.3	0.2	3.1	20.0	28.7	1.4	4.1	3.0	0.4	1.0	0.6	0.1	0.1	3.5	2.3	2.3	16.8	0.5	0.0	0.5	2.4	0.2	3.2	0.0	0.3
LBFDAU seed 71	4.2	0.2	0.1	2.0	22.1	29.1	1.7	4.9	2.3	0.3	0.6	0.6	0.1	0.1	2.9	2.3	2.2	16.7	0.3	0.0	0.7	2.7	0.2	3.3	0.0	0.3
LBFDAU seed 75	4.7	0.2	0.1	2.4	21.4	28.0	2.2	4.8	2.5	0.4	0.7	0.6	0.1	0.1	3.1	2.2	2.4	17.2	0.4	0.0	0.5	2.7	0.2	2.8	0.0	0.3
LBFDAU seed 76	4.7	0.3	0.1	2.1	20.9	28.1	1.0	5.4	3.1	0.6	0.6	0.6	0.1	0.1	2.9	2.1	2.6	16.3	0.3	0.0	0.6	3.4	0.2	3.7	0.0	0.2
LBFDAU seed 77	5.0	0.2	0.1	2.1	18.5	29.7	1.6	4.8	2.9	0.5	0.7	0.6	0.1	0.1	3.9	2.9	2.4	16.7	0.3	0.0	0.5	2.8	0.2	3.3	0.0	0.2
LBFDAU seed 78	4.8	0.2	0.1	1.9	20.1	29.2	1.1	5.1	2.6	0.4	0.6	0.7	0.1	0.1	3.4	2.4	2.7	16.7	0.3	0.0	0.7	3.2	0.2	3.2	0.0	0.2
LBFDAU seed 79	4.3	0.2	0.1	1.9	21.6	28.7	1.5	5.1	2.6	0.4	0.5	0.6	0.1	0.1	3.3	2.3	2.6	16.6	0.3	0.0	0.6	2.9	0.2	3.3	0.0	0.4
LBFDAU seed 80	4.7	0.3	0.2	1.9	19.0	31.0	0.7	4.6	2.7	0.5	0.6	0.6	0.1	0.0	3.3	2.2	2.5	16.1	0.4	0.0	0.6	3.8	0.3	3.7	0.0	0.1
LBFDAU seed 81	4.3	0.2	0.1	2.1	21.0	29.5	1.4	5.1	2.6	0.4	0.6	0.6	0.1	0.1	3.4	2.5	2.3	16.7	0.3	0.0	0.4	3.1	0.1	2.9	0.0	0.2
LBFDAU seed 82	4.5	0.3	0.1	2.0	21.0	29.6	1.4	5.5	2.5	0.5	0.6	0.6	0.1	0.1	2.7	1.9	2.7	16.2	0.3	0.0	0.5	3.2	0.2	3.5	0.0	0.3
LBFDAU seed 83	5.0	0.3	0.1	2.3	20.6	28.3	0.9	5.8	2.8	0.6	0.6	0.6	0.1	0.1	3.0	2.4	2.5	16.2	0.4	0.0	0.5	3.3	0.1	3.3	0.0	0.2
LBFDAU seed 84	6.2	0.5	0.2	2.9	20.3	27.1	0.8	5.6	3.0	0.9	0.9	0.6	0.0	0.0	3.0	2.1	2.1	15.4	0.7	0.0	0.5	3.5	0.0	3.9	0.0	0.0
LBFDAU seed 85	4.1	0.2	0.1	2.2	21.5	30.4	1.4	4.8	2.6	0.4	0.6	0.6	0.1	0.1	3.1	2.4	2.3	16.1	0.4	0.0	0.5	2.6	0.2	3.1	0.0	0.4

LBFDAU seed 86	4.7	0.3	0.2	2.2	20.6	30.6	1.0	5.5	2.8	0.5	0.7	0.6	0.1	0.1	2.7	2.0	2.7	15.5	0.4	0.0	0.5	2.7	0.2	3.2	0.0	0.2
LBFDAU seed 87	4.3	0.3	0.1	2.2	20.9	31.0	1.6	4.5	2.7	0.3	0.6	0.6	0.1	0.1	3.3	2.2	2.1	15.6	0.3	0.0	0.9	2.8	0.2	3.0	0.0	0.3
LBFDAU seed 88	4.1	0.2	0.1	1.9	22.2	30.6	1.3	4.6	2.5	0.5	0.5	0.6	0.1	0.1	2.9	1.7	3.1	15.6	0.3	0.0	0.7	3.0	0.2	2.9	0.0	0.3
LBFDAU seed 89	4.4	0.3	0.1	2.0	21.6	30.5	1.2	5.4	2.3	0.4	0.6	0.6	0.1	0.1	2.9	2.1	2.2	15.3	0.4	0.0	0.7	3.3	0.1	3.1	0.0	0.2
LBFDAU seed 90	4.8	0.2	0.1	2.4	22.3	29.6	1.1	5.2	2.3	0.4	0.7	0.6	0.1	0.1	3.2	2.6	2.2	15.6	0.3	0.0	0.5	2.7	0.1	2.7	0.0	0.3
LBFDAU seed 91	4.8	0.2	0.1	2.2	22.0	30.2	1.3	4.9	2.7	0.6	0.7	0.7	0.1	0.0	3.2	2.2	2.5	15.1	0.3	0.0	0.4	2.5	0.2	3.0	0.0	0.3
LBFDAU seed 92	5.2	0.3	0.2	2.0	20.3	29.7	1.1	5.1	3.2	0.6	0.6	0.6	0.1	0.0	3.1	1.9	3.4	14.8	0.4	0.0	0.6	3.3	0.2	3.2	0.0	0.2
LBFDAU seed 93	4.7	0.2	0.1	2.4	23.0	30.3	1.1	5.3	2.1	0.5	0.7	0.7	0.1	0.1	3.0	2.2	2.2	15.1	0.3	0.0	0.4	2.7	0.1	2.5	0.0	0.3
LBFDAU seed 94	5.0	0.3	0.2	2.3	21.9	30.6	1.3	5.2	2.4	0.7	0.8	0.6	0.1	0.1	2.9	2.1	2.4	14.7	0.4	0.0	0.4	2.7	0.2	2.8	0.0	0.3
LBFDAU seed 95	4.8	0.2	0.1	2.5	24.6	29.6	1.3	5.2	2.1	0.5	0.7	0.7	0.1	0.1	3.2	2.2	2.4	13.5	0.3	0.0	0.5	2.2	0.1	2.5	0.0	0.3

Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in greenhouses during the summer

The data in Table 148 indicate the copy number of the selected events was a single insertion which was homozygous in the T3 seed. Fatty acid profile measurements, see Table 149 and Table 150, indicated the combination of T-DNA from VC-LTM593-1qcz rc are capable of bringing in the VLC-PUFA pathway to successfully accumulate ARA, EPA and DHA. The data on Table 151 show that there was no significant impact on the aerial portion of the plant caused by VC-LTM593-1qcz rc.

Table 148: Copy number measurement of T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T2 plants that where measured per event. For each event, T2 seedbatches of two homozygous T1 plants where selected for seeding. Comparison of the results with Table 135 confirmes homozygozity of all T2 plants.

Event	Copy number assays targeting the T-DNA of VC-LTM593-1qcz rc. Assays are listed according to the position of the assay target along the T-DNA, with target c-AHAS located near the left T-DNA border and target c-d6Elo(Pp_GA) near the right T-DNA border.															
	c-AHAS	j-i-Atss1_c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	j-p-LuPXR_i-Atss15	j-p-PvARC5_t-BnSETL	c-d5Des(Tc_GA)	j-i-Atss18_c-o3Des(Pi_GA2)	c-o3Des(Pi_ GA)	j-p-BnSETL-v1_c-o3DES(Pir_GA)	j-i-Atss14_c-d12Des(Ps_GA)	c-d6Elo(Tp_GA)	j-t-StCAT_p2_p-LuPXR	j-i-Atss2_c-d6Des(Otfebit_GA)	c-d5Des(Tc_GA)	c-d6Elo(Pp_GA)	j-i-Atss18_c-d6Elo(Pp_GA2)
LBFDAU (n=218)	2.0	2.1	4.1		3.8	8.1	4.0	4.0			4.1	4.2	4.0	8.1	4.1	4.0
LBFDGG (n=200)	2.0	2.0	2.0		2.0	4.0	2.0	2.0			2.1	2.1	2.0	4.0	2.2	2.1
LBFGKN (n=182)	2.0	2.0	2.0		2.0	4.2	2.1	1.9			2.0	2.2	2.0	4.2	2.1	2.1
LBFIHE (n=157)	2.1	2.0	2.0		2.1	4.3	2.0	2.0			2.2	2.1	2.0	4.3	2.1	2.0
LBFLDI (n=229)	2.3	2.2	2.3		2.3	4.7	2.3	2.3			2.4	2.4	2.3	4.7	2.4	2.2
LBFPRA (n=196)	2.0	1.9	5.8		3.9	7.9	4.0	4.1			3.9	4.0	3.9	7.9	4.0	1.9
LBFDHG (n=177)	4.1	4.2	4.2		4.0	8.3	4.1	4.1			4.2	4.1	4.0	8.3	4.1	4.0
LBFLFK (n=195)	4.0	4.1	4.0		4.0	8.2	4.0	4.0			4.0	4.0	4.0	8.2	4.0	4.0

Table 149: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T3 seed batches representing a plant measured per event. Per seed batch a random selection of ~15 seed was measured in five technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b

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LBFDAU (n=218)	4.8 ± 0.2	0.2 ± 0	0 ± 0	4.2 ± 0.3	23 ± 1.6	31.5 ± 1.2	1.1 ± 0.2	4.6 ± 0.4	2.2 ± 0.4	0.4 ± 0.1	1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.5 ± 0.8	3.2 ± 0.6	1.4 ± 0.3	10.5 ± 1.4	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.3 ± 0.3	0.2 ± 0.1	2.1 ± 0.4	0.6 ± 0.2	0.4 ± 0.1
LBFDGG (n=200)	5 ± 0.2	0.2 ± 0	0 ± 0	3.2 ± 0.3	30.4 ± 2.2	34.1 ± 1.6	0.8 ± 0.1	5 ± 0.5	1.6 ± 0.2	0.3 ± 0	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	2.4 ± 0.2	1.4 ± 0.2	2 ± 0.3	7 ± 0.8	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.2	0.1 ± 0	1.3 ± 0.3	0.3 ± 0	0.1 ± 0
LBFGKN (n=182)	4.8 ± 0.2	0.2 ± 0	0 ± 0	3.6 ± 0.4	28.3 ± 1.9	34.6 ± 1.6	0.7 ± 0.2	4.7 ± 0.5	1.8 ± 0.4	0.3 ± 0.2	0.9 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0	2.4 ± 0.3	1.3 ± 0.2	2.4 ± 0.3	7.9 ± 0.9	0.4 ± 0	0 ± 0	0.4 ± 0.1	2 ± 0.2	0.1 ± 0.1	1.6 ± 0.3	0.3 ± 0.1	0.2 ± 0.1
LBFIHE (n=157)	4.9 ± 0.3	0.2 ± 0	0 ± 0	3.7 ± 0.6	25.9 ± 1.9	32.9 ± 1.8	0.8 ± 0.1	4.7 ± 0.6	2.4 ± 0.7	0.3 ± 0.1	0.8 ± 0.1	0.7 ± 0	0.2 ± 0.1	0.1 ± 0.1	2.7 ± 0.3	1.7 ± 0.3	2.5 ± 0.4	9.6 ± 1.3	0.4 ± 0	0 ± 0	0.4 ± 0.1	2.2 ± 0.3	0.2 ± 0.1	2 ± 0.5	0.3 ± 0.1	0.3 ± 0.1
LBFLDI (n=229)	5.9 ± 1	0.3 ± 0.1	0 ± 0.1	2.9 ± 0.3	27.8 ± 2.5	32.5 ± 1.8	0.7 ± 0.1	4.7 ± 0.5	1.7 ± 0.3	0.4 ± 0.1	0.8 ± 0.1	0.6 ± 0.1	0.1 ± 0	0.1 ± 0	2.4 ± 0.3	1.4 ± 0.2	2.4 ± 0.4	9.2 ± 1.5	0.3 ± 0	0 ± 0	0.5 ± 0.1	2.6 ± 0.4	0.1 ± 0	1.7 ± 0.4	0.3 ± 0.1	0.2 ± 0
LBFPRA (n=196)	5 ± 0.2	0.2 ± 0	0 ± 0	3.9 ± 0.7	23.2 ± 2	30.9 ± 1.9	1.1 ± 0.2	3.8 ± 0.6	2.6 ± 0.5	0.3 ± 0.1	0.9 ± 0.1	0.6 ± 0	0.1 ± 0	0.1 ± 0	3 ± 0.4	1.7 ± 0.2	3.5 ± 0.4	13.5 ± 2	0.4 ± 0	0 ± 0	0.3 ± 0.1	2.6 ± 0.3	0.1 ± 0.1	1.7 ± 0.3	0.1 ± 0	0.3 ± 0.1
LBFDHG (n=177)	5.7 ± 0.2	0.3 ± 0	0 ± 0	3.2 ± 0.5	24.1 ± 0.8	34.5 ± 1.4	0.8 ± 0.1	4.4 ± 0.3	2.5 ± 0.5	0.3 ± 0.1	0.9 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.1 ± 0.6	1.9 ± 0.4	1.5 ± 0.3	8.5 ± 1.2	0.4 ± 0	0 ± 0	0.8 ± 0.1	2.5 ± 0.3	0.2 ± 0.1	2.4 ± 0.3	0.8 ± 0.2	0.2 ± 0.1
LBFLFK (n=195)	5 ± 0.1	0.2 ± 0	0.1 ± 0.1	4 ± 0.4	25.9 ± 1.8	31.9 ± 1.3	1 ± 0.2	4.7 ± 0.4	1.8 ± 0.3	0.4 ± 0.1	1 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4 ± 0.9	2.4 ± 0.5	1.4 ± 0.3	8.3 ± 1	0.4 ± 0	0 ± 0	0.6 ± 0.1	2.7 ± 0.5	0.2 ± 0	1.5 ± 0.4	0.8 ± 0.3	0.3 ± 0.1

Table 150: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU (n=1)	5.1	0.3	0.0	4.7	20.6	27.6	1.1	4.3	2.5	0.5	1.3	0.6	0.2	0.1	4.0	2.8	1.6	14.1	0.5	0.0	0.4	2.8	0.3	3.5	0.5	0.3
LBFDGG (n=1)	5.2	0.3	0.0	2.9	25.6	33.8	0.7	5.2	2.0	0.4	0.8	0.7	0.2	0.1	2.5	1.6	2.9	9.1	0.4	0.0	0.5	2.4	0.2	2.2	0.3	0.1
LBFGKN (n=1)	4.8	0.2	0.0	3.7	26.5	30.2	1.2	4.9	2.3	0.3	0.8	0.6	0.2	0.1	2.5	1.4	3.2	10.9	0.3	0.0	0.4	2.4	0.1	2.5	0.2	0.3
LBFIHE (n=1)	4.4	0.1	0.0	3.0	20.2	29.0	0.8	4.7	2.8	0.5	0.7	0.7	0.4	0.3	2.9	2.1	3.3	15.3	0.3	0.0	0.5	2.9	0.4	3.9	0.2	0.5
LBFLDI (n=1)	6.7	0.3	0.0	3.0	22.2	28.3	1.1	4.0	2.7	0.7	0.8	0.5	0.1	0.1	3.2	2.1	2.5	13.8	0.4	0.0	0.5	3.4	0.3	2.7	0.5	0.2
LBFPRA (n=1)	5.0	0.2	0.0	3.5	20.0	27.6	1.4	3.1	3.4	0.4	0.8	0.6	0.1	0.1	2.9	1.9	3.8	19.0	0.3	0.0	0.3	2.7	0.1	2.4	0.1	0.4
LBFDHG (n=1)	6.0	0.3	0.0	2.6	23.3	29.4	1.1	5.0	2.3	0.4	0.8	0.7	0.1	0.1	2.8	1.9	1.6	12.8	0.4	0.0	0.7	3.3	0.4	3.3	0.6	0.3
LBFLFK (n=1)	4.9	0.2	0.2	3.8	23.0	31.9	1.0	4.4	2.3	0.5	0.9	0.7	0.1	0.1	3.2	2.1	2.0	11.6	0.3	0.0	0.7	3.0	0.2	2.3	0.5	0.3

Table 151: Phenotypic rating of T1 plants cultivated in the greenhouse of canola events containing the T-DNAs of plasmids VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T1 plants that where rated per event. DFF: days to first flower (days), DF: deformed flower (1=deformed, 9=normal), DL: deformed leaf (1=deformed, 9=normal), DP: deformed plant (1=deformed, 9=normal), DS: deformed silique (1=deformed, 9=normal), FC: flower color (1=white, 3=optimal, 4=orange/yellow), LD: leaf dentation (3=no dentation, 7=strong dentation), LGC: leaf color (3=yellow, 5=optimal, 7=blueish), LF: fertility (1=low, 9=very high), NoL: number of lobes (#), PH: plant height (cm), TKW: thousand kernel weight (g), SC: seed quality (1=good, 9=bad), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil)

Event	DFF	DF	DL	DP	DS	FC	LD	LGC	LF	NoL	PH	TKW	SC	Oil	Protein
LBFDAU (n=218)	43.2 ± 2.9	9 ± 0	9 ± 0	8 ± 0	9 ± 0	3 ± 0	4 ± 0.1	5 ± 0	8.6 ± 0.8	5.1 ± 0.6	107.5 ± 7.6	3 ± 0.3	3.7 ± 1.2	35.6	
LBFDGG (n=200)	43 ± 3.7	8.6 ± 1.5	8.5 ± 0.5	8 ± 0	8.6 ± 1.3	3 ± 0	4 ± 0	5 ± 0	8.7 ± 0.7	4.5 ± 0.5	112.1 ± 5.5	3 ± 0.3	3.7 ± 0.7	N/A	
LBFGKN (n=182)	42.5 ± 4.3	8 ± 2.7	8.5 ± 1	7.6 ± 1.6	8.1 ± 1.9	2.9 ± 0.3	4.1 ± 0.8	5 ± 0.3	8.5 ± 1	5 ± 0	111.6 ± 15.2	3.3 ± 0.4	3.3 ± 0.6	36.1	
LBFIHE (n=157)	51.1 ± 5.2	8.9 ± 0.4	8.9 ± 0.5	8.9 ± 0.5	5.2 ± 2.6	3 ± 0	5.1 ± 0.4	4.9 ± 0.4	5.2 ± 2.6	6.6 ± 1.1	139.4 ± 14.7	4 ± 0.5	3.8 ± 1.9	34.1	
LBFLDI (n=229)	48.6 ± 4.5	9 ± 0	9 ± 0	9 ± 0	7.6 ± 2.5	3 ± 0	5 ± 0.1	5 ± 0.1	7.6 ± 2.5	6.2 ± 1.1	145.5 ± 15.9	4.2 ± 0.4	3.1 ± 1.3	N/A	
LBFPRA (n=196)	51.2 ± 2.4	9 ± 0	9 ± 0	9 ± 0	5.8 ± 3.2	3 ± 0	5 ± 0	5 ± 0	5.8 ± 3.2	7.3 ± 1	138.7 ± 16.6	4.2 ± 0.4	2.4 ± 1.5	34.7	
LBFDHG (n=177)	46 ± 3.5	9 ± 0	9 ± 0	8 ± 0	4.7 ± 1.6	3 ± 0	4 ± 0	5 ± 0	8.5 ± 0.8	5.2 ± 0.8	112.5 ± 5.4	3.4 ± 0.4	5.3 ± 1	32.3	
LBFLFK (n=195)	42.3 ± 2.8	9 ± 0	9 ± 0	9 ± 0.3	8.9 ± 0.7	3 ± 0	4 ± 0.1	5 ± 0	8.7 ± 0.9	5.3 ± 0.5	113.4 ± 8	3.3 ± 0.3	3.9 ± 1.5	37.1	

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Fatty acid profiles, copy number measurements, and phenotypic observations of T2 plants carrying T-DNAs of plasmids VC-LTM593-1qcz rc cultivated in field trials in USDA growth zones 3a-4b and 5a during the summer

Field data for the T3 seed from the events carrying the T-DNA from VC-LTM593-1qcz rc, shown in Table 152 and Table 153, indicate that the plants are capable of making VLC-PUFAs in the field (ARA, EPA and DHA), though not at the level observed in the greenhouse. ANOVA was conducted with using the software JMP 11.0. Analysis was conducted at the 95% confidence level using Tukey test. To compensate for unbalance in the data obtained from the field trial (e.g. due to e.g. weather), Least Square menas instead of means where used in the statistical analysis. Common letters in the Table 154, Table 155and Table 156 indidcate no significant difference of the least square means. Table 154 shows the statistical analysis of agronomical parameters.

There was a difference in seed oil content observed compared to the greenhouse (e.g. comparing Table 154 with Table 151), indicating oil content and the fatty acid profile could be linked. These observations are in agreement with previous examples where it was observed that increased oil contents in the field grown plants concomitant with a decrease in VLC-PUFAs, in particular EPA, DHA and ARA. A more detailed description of the observations regarding oil content and VLC-PUFAs is given in Example 20.

For seed yield (kg per ha, data not shown), no statisticaly relevant difference was found comparing the events against wildtype Kumily (tested using Tukey, 0.05% level).

Table 152: Fatty acid profiles of T3 seeds harvested from T2 cultivated in the field, corresponding to USDA growth zones 3a-4b and 5a, for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of T3 seed aliquots representing a plot where measured per event. For event LBFGKN, 36 plots and 60 single plants from those plots where measured. Per seed batch a random selection of ~15 seed was measured in five technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU (n=16)	4.7 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.1	28.6 ± 1.5	29.2 ± 0.7	1 ± 0.1	6.1 ± 0.3	1.6 ± 0.1	0.3 ± 0	0.7 ± 0	0.7 ± 0	0.1 ± 0	0.1 ± 0	3.3 ± 0.3	2.2 ± 0.2	2 ± 0.2	10.7 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	2.9 ± 0.2	0.1 ± 0	1.6 ± 0.2	0.3 ± 0.1	0.3 ± 0
LBFDGG (n=36)	4.7 ± 0.1	0.2 ± 0	0 ± 0	2.5 ± 0.2	34.2 ± 1.9	32.3 ± 1.2	0.6 ± 0.1	7 ± 0.5	1.2 ± 0.1	0.2 ± 0	0.6 ± 0	0.8 ± 0	0.1 ± 0	0.1 ± 0	2 ± 0.3	1.3 ± 0.2	1.9 ± 0.2	6.1 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	2.1 ± 0.2	0.1 ± 0	1.1 ± 0.2	0.2 ± 0.1	0.1 ± 0
LBFGKN (n=36+60)	4.6 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	33.7 ± 1.7	32.8 ± 1.4	0.6 ± 0.1	7.5 ± 0.6	0.9 ± 0.1	0.2 ± 0	0.7 ± 0	0.8 ± 0.1	0.2 ± 0	0.1 ± 0	2.1 ± 0.3	1.2 ± 0.1	1.8 ± 0.2	6 ± 0.6	0.3 ± 0	0 ± 0	0.3 ± 0.1	2.1 ± 0.2	0.1 ± 0	1 ± 0.1	0.2 ± 0	0.2 ± 0
LBFIHE (n=36)	4.8 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	31.2 ± 1.7	33.9 ± 1.2	0.6 ± 0.1	6.7 ± 0.7	1.3 ± 0.2	0.3 ± 0	0.7 ± 0.1	0.8 ± 0	0.2 ± 0	0.1 ± 0	2.1 ± 0.2	1.2 ± 0.1	2.4 ± 0.3	6.7 ± 0.6	0.3 ± 0	0 ± 0	0.3 ± 0.1	1.9 ± 0.2	0.1 ± 0	1.2 ± 0.2	0.2 ± 0.1	0.2 ± 0
LBFLDI (n=36)	4.9 ± 0.2	0.3 ± 0	0 ± 0	2.5 ± 0.2	33.4 ± 1.7	32.7 ± 1.2	0.6 ± 0.1	6.8 ± 0.6	1 ± 0.1	0.2 ± 0	0.7 ± 0.1	0.8 ± 0	0.2 ± 0	0.1 ± 0	2 ± 0.3	1.2 ± 0.2	2 ± 0.2	6.2 ± 0.7	0.3 ± 0	0 ± 0	0.3 ± 0	2.1 ± 0.2	0.1 ± 0	1.1 ± 0.2	0.2 ± 0	0.2 ± 0
LBFIDT (n=32)	4.6 ± 0.1	0.2 ± 0	0 ± 0	2.7 ± 0.2	30 ± 1.4	29.9 ± 1	0.9 ± 0.1	6.5 ± 0.5	1.6 ± 0.1	0.3 ± 0	0.7 ± 0.1	0.7 ± 0	0.1 ± 0	0.1 ± 0	4.4 ± 0.4	2.6 ± 0.2	1.5 ± 0.2	6.8 ± 0.7	0.3 ± 0	0 ± 0	0.6 ± 0.1	2.9 ± 0.3	0.1 ± 0	1.2 ± 0.2	1 ± 0.1	0.3 ± 0
LBFPRA (n=36)	4.8 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	28.4 ± 2.1	32.7 ± 1.4	0.8 ± 0.1	5.7 ± 0.4	1.6 ± 0.2	0.3 ± 0.1	0.7 ± 0	0.8 ± 0	0.2 ± 0	0.1 ± 0	2.3 ± 0.3	1.2 ± 0.2	3.8 ± 0.5	9.6 ± 1	0.3 ± 0	0 ± 0	0.3 ± 0	2.4 ± 0.3	0.1 ± 0	1.1 ± 0.2	0.1 ± 0	0.2 ± 0.1
LBFDHG (n=4)	5.2 ± 0.1	0.2 ± 0	0 ± 0	2.4 ± 0.1	28.2 ± 1.4	29.5 ± 1	0.9 ± 0.2	6.5 ± 0	1.6 ± 0.1	0.3 ± 0	0.6 ± 0	0.7 ± 0	0.2 ± 0	0.1 ± 0	2.9 ± 0	1.7 ± 0	2 ± 0.1	9.6 ± 0.4	0.3 ± 0	0 ± 0	0.6 ± 0	3.5 ± 0.1	0.2 ± 0	1.9 ± 0.2	0.5 ± 0.1	0.3 ± 0
LBFLFK (n=36)	4.7 ± 0.2	0.2 ± 0	0 ± 0	2.6 ± 0.2	30.1 ± 1.9	30.2 ± 1.1	0.9 ± 0.1	6.2 ± 0.4	1.5 ± 0.2	0.3 ± 0.1	0.6 ± 0	0.8 ± 0	0.1 ± 0	0.1 ± 0	3.3 ± 0.3	1.9 ± 0.2	1.9 ± 0.2	8.2 ± 1	0.3 ± 0	0 ± 0	0.5 ± 0	3.2 ± 0.4	0.1 ± 0	1.4 ± 0.3	0.5 ± 0.1	0.3 ± 0.1

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Table 153: Fatty acid profiles of one T3 seed batch per event harvested from T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. Fatty acid profiles of T3 seed batches representing a field plot having the highest EPA+DHA levels per event are shown. Per seed batch, a random selection of ~30 seed was measured in two technical repeats.

Event	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9	22:4 n-6	22:5 n-3	22:5 n-6	22:6 n-3	unknown peak a	unknown peak b
LBFDAU (n=1)	4.9	0.2	0.0	2.6	26.0	28.9	0.8	6.2	1.7	0.3	0.7	0.8	0.2	0.1	3.6	2.4	2.0	12.0	0.3	0.0	0.3	3.2	0.1	2.1	0.4	0.3
LBFDGG (n=1)	5.0	0.3	0.0	2.7	30.1	33.1	0.5	6.5	1.5	0.3	0.7	0.8	0.2	0.1	2.1	1.4	2.4	7.5	0.3	0.0	0.4	2.4	0.1	1.6	0.2	0.1
LBFGKN (n=1)	4.4	0.2	0.0	2.6	32.6	31.1	0.7	7.3	1.1	0.3	0.6	0.8	0.2	0.1	2.1	1.3	2.1	7.7	0.3	0.0	0.3	2.5	0.1	1.3	0.2	0.2
LBFIHE (n=1)	4.8	0.2	0.0	2.5	28.0	33.9	0.6	6.3	1.6	0.3	0.8	0.8	0.2	0.1	2.4	1.5	2.7	8.2	0.3	0.0	0.4	2.3	0.1	1.6	0.2	0.2
LBFLDI (n=1)	5.2	0.3	0.0	2.5	29.8	33.3	0.4	6.8	1.3	0.4	1.1	0.8	0.2	0.1	1.8	1.2	2.4	7.2	0.4	0.0	0.4	2.4	0.1	1.6	0.2	0.2
LBFIDT (n=1)	4.7	0.2	0.0	2.6	28.6	28.8	1.0	7.1	1.6	0.3	0.7	0.7	0.1	0.1	4.3	2.6	1.5	8.1	0.3	0.0	0.6	3.4	0.1	1.4	1.0	0.4
LBFPRA (n=1)	5.1	0.2	0.0	2.6	26.0	31.5	0.7	5.3	1.8	0.3	0.7	0.7	0.2	0.1	2.4	1.3	4.3	11.7	0.3	0.0	0.3	2.8	0.0	1.3	0.1	0.2
LBFDHG (n=1)	5.1	0.2	0.0	2.4	29.0	28.4	1.0	6.6	1.6	0.3	0.6	0.8	0.1	0.1	2.9	1.7	1.9	10.2	0.3	0.0	0.5	3.5	0.2	1.9	0.5	0.3
LBFLFK (n=1)	5.1	0.2	0.0	2.6	26.9	29.3	0.8	5.9	1.9	0.4	0.7	0.8	0.1	0.1	3.1	2.0	2.3	10.3	0.3	0.0	0.6	3.9	0.1	2.0	0.5	0.2

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Table 154: Phenotypic rating of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column, along with the number of field plots that where rated per event. Stand: the number of plants that emerged within a 1 meter section of row at GS 14 (4 leaves unfolded), rating of a seedlings ability to grow and develop at GS 14 (4 leaves unfolded, 1= All plants at different growth stages and all plants appear unhealthy, 9= All plants at same growth stage and all plants appear healthy), First flower: date at which plants are at GS 60 (days after emergence), Last flower: date at which plants are at GS 69 (days after emergence), Maturity: Days to Maturity (when average seed moisture is 30 to 35%, and 30 to 40% if the seeds in the pods have developed their mature seed color, either black or yellow),Plant Heigth: the average height (cm) of five plants in a plot measured from soil level to the top of the plant at GS 69 (cm), Lodging: rating of the average standability of plants in a plot at GS 83 (30% of pods ripe, 1=0 – 10% average lean of plants in plot from horizontal (standing erect), 9=81%+ average lean of plants in plot from horizontal (nearly prostrate)), Shatter: rating of the ability of plants to retain seed in the pod at GS 89 (all pods fully ripe, 1=0 – 10% perished pods, 9=81%+ perished pods), Agron Score: scale of 1-5 with 1 being the best, 5 the worst ranking of plant phenotype (measurements occurred post flower during pods formation and seed development),Moisture (% of seed weight), TKW: thousand kernel weight (g), Oil: oil content (% of seed weight), protein: Protein content (% of seed cake without oil).

Event	Stand		Vigor		First Flower		Last Flower		Maturity		Plant Height		Lodging		Shatter		Agron Score		Moisture		TWK		Oil		Protein	
LBFDAU (n=16)	23.7	a	5.7	bcd	43.8	bc	77.3	abcde	94.5	a	115.5	b	2.3	ab	1.5	a	4.7	ab	9.0	a	3.5	abcd	38.5	bc	26.6	c
LBFDGG (n=36)	23.0	a	5.9	bcd	44.0	bc	77.1	bcd	94.3	a	116.2	b	2.3	b	1.6	a	4.2	ab	8.9	a	3.8	a	38.8	abc	26.3	c
LBFDHG (n=4)	23.4		5.8		43.9		77.2				115.9		2.3		1.5		4.4		9.0		3.7		39.3	abc	26.5	c
LBFGKN (n=36)	22.8	a	6.3	bcd	43.3	c	76.5	cde	94.2	a	118.8	b	2.8	ab	1.8	a	4.1	ab	8.5	a	3.7	ab	36.7	c	26.5	c
LBFIHE (n=36)	21.7	a	5.6	cd	45.7	b	79.7	ab	94.7	a	115.2	b	2.3	b	1.6	a	4.1	ab	8.7	a	3.4	bcd	39.8	ab	26.5	c
LBFLFK (n=36)	22.5	a	5.1	d	45.6	b	78.0	abc	94.3	a	118.4	b	2.6	ab	1.5	a	4.1	ab	8.6	a	3.7	a	38.3	bc	27.1	c
LBFPRA (n=36)	22.5	a	6.3	bcd	44.2	bc	78.1	abc	94.3	a	121.2	b	2.9	ab	1.9	a	4.2	ab	8.5	a	3.5	abc	39.2	abc	26.5	c
Topas	24.8	a	5.2	d	48.1	a	80.9	a	95.3	a	139.7	a	3.2	ab	2.6	a	4.4	a	7.3	b	3.3	cd	37.8	c	26.9	c
Kumily	28.2	a	6.9	ab	43.4	c	76.6	cde	94.2	a	119.9	b	2.8	ab	1.8	a	4.5	a	7.4	b	3.7	ab	39.8	ab	28.5	a
Control 1	28.1	a	7.7	a	42.9	c	73.8	e	89.2	b	121.1	b	3.9	a	2.7	a	3.4	b	7.0	b	3.2	d	39.9	ab	27.1	c
Control 2	25.3	a	6.6	abc	40.7	d	74.3	de	90.3	b	119.1	b	3.8	a	2.7	a	3.4	b	7.0	b	3.1	d	40.7	a	27.4	bc

Table 155: Compositional analysis of T3 seeds of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-LTM593-1qcz rc. The events are indicated in the first column. The analysis has been done on 4 BULK, whereby each BULK is a representative sample of all seeds harvedted from 4 different geographic reagions. Alpha-Tocopherol (mg/100g seed), Beta-Tocopherol (mg/100g seed), Delta-

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Tocopherol (mg/100g seed), Gamma-Tocopherol (mg/100g seed), Tocopherol (mg/100g seed), Sinapine (µg/g (ppm)), Phytate (% of seed weight (w/w)), Ash (% of seed weight (w/w)), Crude Fiber (% of seed weight (w/w)), ADF: acid detergent fiber (% of seed weight (w/w)), NDF: neutral detergent fiber (% of seed weight (w/w)). All results have been normalized to the seed weight of seeds having 0% moisture.

Event	Alpha-Tocopherol		Beta-Tocopherol		Delta-Tocopherol		Gamma-Tocopherol		Tocopherols (VitE)		Sinapine		Glucosinolate		Phytate		Ash		Crude Fiber		ADF		NDF	
LBFDAU	13.3	ab	0.25	a	0.58	a	29.5	a	43.7	a	0.64	cd	11.0	bcd	2.3	B	4.5	ab	9.4	a	11.8	ab	14.5	a
LBFDGG	14.1	ab	0.23	a	0.45	bcd	25.6	b	40.4	abc	0.69	bcd	13.1	ab	2.3	B	4.3	ab	9.2	a	10.9	cdef	13.6	ab
LBFGKN	12.9	b	0.23	a	0.52	abc	26.9	ab	40.6	abc	0.70	bc	11.3	bc	2.3	b	4.4	ab	9.3	a	11.0	bcdef	13.5	ab
LBFIHE	13.2	ab	0.23	a	0.45	bcd	22.0	cd	35.9	cde	0.65	cd	15.7	a	2.2	b	4.7	ab	9.1	a	10.8	cdef	13.4	ab
LBFLFK	12.5	b	0.23	a	0.52	abc	25.7	b	38.9	abc	0.68	bcd	12.6	abc	2.3	b	4.3	b	9.6	a	11.3	abcde	13.7	ab
LBFPRA	13.6	ab	0.22	a	0.47	bcd	24.9	bc	39.2	abc	0.62	d	10.9	bcd	2.2	b	4.3	ab	9.6	a	12.0	a	14.6	a
Topas	14.7	ab	0.25	a	0.36	d	16.6	e	31.9	e	0.69	bcd	7.6	d	2.7	a	4.8	a	9.0	a	10.5	ef	12.9	b
Kumily	12.3	b	0.23	a	0.54	ab	24.4	bc	37.5	bcd	0.78	a	11.0	bcd	2.2	b	4.3	b	9.1	a	11.5	abc	14.3	a
Control 1	16.6	a	0.25	a	0.43	cd	24.1	bc	41.4	ab	0.73	ab	9.5	cd	2.3	b	4.6	ab	8.6	a	10.2	f	12.8	b
Control 2	12.0	b	0.20	a	0.45	bcd	20.8	d	33.5	de	0.72	ab	11.9	bc	2.3	b	4.4	ab	9.2	a	10.6	def	13.8	ab

Table 156: Herbicde tolerance of T2 plants cultivated in USDA growth zones 3a-4b and 5a for field trials of canola events containing the T-DNAs of plasmid VC-VC-LTM593-1qcz rc. The events are indicated in the first column. IMI Injury: injury according to the scale detailed in Table 157 (DAT= days after treatment). Herbicide imazamox was aplied at a 2x rate of 70 g imazamox /ha. Brassica napus cv Kumily, which is the non-transgeneic comparator line that is otherwise isogenic to the events, was rated at 6 to 7, and was removed from the statistical analysis to make the Tukey test more sensitive to detect significant differences between events that are very similar in their tolerance.

Event	IMI Injury 7 DAT		IMI Injury 14 DAT		IMI Injury 21 DAT	
LBFDAU	2	a	1	ab	1	a
LBFDGG	2	a	1	ab	1	a
LBFDHG	2		1		1	
LBFGKN	2	a	1	ab	1	a
LBFIHE	2	a	2	a	1	a
LBFLFK	2	a	1	b	1	a

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Event	IMI Injury 7 DAT		IMI Injury 14 DAT		IMI Injury 21 DAT	
LBFPRA	2	a	1	ab	1	a
Topas	1	a	1	b	1	a
Kumily	6		6		7	

Table 157: Canola rating scale for herbicide

% Injury	1-7 Scale	Category	Injury Symptoms	Growth Rates and Recovery Effects
0	1	Excellent	None	None
1-6	2	Very Good	Leaf and petiole epinasty, chlorosis.	Minor or temporary growth effects. Injury and effects should be minor enough to not cause commercialization concerns.
7-14	3	Good	Leaf, petiole and stem epinasty, chlorosis, stem swelling. Leaf cupping may be observed.	This would be the maximum allowable injury for commercial evaluations. Fairly temporary in nature without any effect on final yield and minimal delay in maturity,
15-20	4	Fair	Above symptoms plus stunting in height, smaller leaf size or impact on LAI, in this class: Basal swelling may be observed. Expect recovery and seed production with this set of symptoms but delayed, reduced growth and reduced seed set. Plant stand may be non-uniform upon recovery.	Appearance of unaffected new growth impeded for <7 days. Slight delay in bolting and flower production. Yield impact minimal or small at harvest.
21-40	5	Poor	Injury in this class would be as above and more than evaluator's estimate of the level of commercial acceptance.	Significant delay in plant development, significant malformation s in growth and development vs. control. Malformations persist Serious reduction in maturity, height and harvest yield.
41-79	6	Non Tolerant		Equivalent to suppression as a volunteer crop in a weed control assessment. Minimal regrowth following application. Plants survive but fail to flower and mature as normal.
80-100	7	Susceptible	Severe injury or death.	Severe injury or death.

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Example 19: Gene copy number effects on observed conversion efficiencies at each pathway step.

When analysing the VLC-PUFA levels of the various constructs listed in this invention, distinct differences were observed in conversion efficiency at the pathway steps when the T-DNA copy number increased. This was observed when comparing the VCL-PUFA levels of (1) events having a single copy T-DNA insertion vs a double copy T-DNA insertion, (2) heterozygous plants vs homozygous plants, and (3) when analysing segregating single seeds as described in Example 9 and shown in Figure 22 and Figure 23. This demonstrates a relationship between 'gene dosage', that is the number of T-DNA copies present in the genome, and VLC-PUFA levels. Upon further investigation it is clear that certain genes/steps of the VLC-PUFA pathway benefit more in terms of impact on the pathway when the gene number and/or expression level of the gene or activity at that step is increased. While the contribution from each gene present, when multiple genes which encode enzymes with identical activities are present, are difficult to assess (e.g. two different omega-3-desaturases are technically difficult to assess), it was possible to calculate conversion efficiencies for each pathway step, by using the equations shown in Figure 2. These conversion efficiencies for each pathway step were calculated for various populations of plants that represent a certain gene dosage, in order to investigate if the observed increase in conversion efficiency can be assigned to one or more individual pathway steps (e.g. increasing conversion efficiency due to increased gene dosage at one early step in the pathway may increase conversion efficiency at a later step simply be providing more of a limiting substrate).

The conversion efficiencies are sometimes referred to as "apparent" conversion efficiencies because for some of the calculations it is recognized that the calculations do not take into account all factors that could be influencing the reaction. As an example of factors that may be influencing a reaction but are not taken into account during the conversion efficiency calculation; the substrates of the delta-6-desaturase are produced while bound at the sn-2 positions of phospholipids, whereas the product of the delta-6-desaturase is formed while the fatty acid is bound to CoA. It is therefore not immediately clear if the low delta-6-conversion conversion efficiencies shown in Figure 33 are due to inefficient provision of substrate to the delta-6-desaturase via trans-esterification from PC (phosphatidylcholine) bound LA/ALA to CoA bound LA/ALA, or whether the delta-6-desature had low activity/low conversion efficiency.

For the interpretation of the conversion efficiencies it was also important to note that any catalyzed conversion of substrate to product was dependent on the substrate concentration, catalyst (enzyme) concentration, and product concentration.

Analysing the data pathway step by pathway step, the following observations can be made in Figure 32 to Figure 39:

Conversion efficiency of delta-12-desaturation

The delta-12-desaturase conversion efficiency encompasses all steps that influence substrate concentration, product concentration, and enzyme concentrations. From the data in example 21 it is evident that the delta-12-desaturase, c-d12Des(Ps_GA), enzymatically produces phosphatidylcholine bound 18:2n-6 (Figure 26, Panel A), which implies the product was phosphatidylcholine bound 18:1n-9. The primary flux of newly formed 18:1n-9 was derived from plastid produced 18:1n-9, which was exported into the cytosol, where it was bound to CoA. Therefore, a prerequisite of delta-12-desaturation was the incorporation of CoA-bound 18:1n-9 into the sn2 position of phosphatidylcholine via LPCAT (Lysophosphatidylcholine acetyltransferase EC 2.3.1.23), or via LPAAT (Lysophosphatidic acid acyltransferase E.C. 2.3.1.51), with subsequent conversion of the formed (sn2)18:1n-9-phosphatidic acid into (sn2)18:1n-9-DAG (DAG is an abbreviation for diacylglycerol), whereby (sn2)18:1n-9-DAG was either directly converted into (sn2)18:1n-9-PC (PC is an abbreviation for phosphatidylcholine) substrate for the delta-12-desaturases via PDCT (phosphatidylcholine diacylglycerol cholinephosphotransferase EC 2.7.8.2), or obtains a phosphocholine headgroup via CPT (CPT is an abbreviation for sn-1,2-diacylglycerol:cholinephosphotransferase EC 2.7.8.2) to be converted into (sn2)18:1n-9-PC. The efficiency of this incorporation of plastid synthesized 18:1n-9 directly affects delta-12-desaturase substrate concentration. As mentioned already, PC is converted by PDCT into DAG (demonstrated by Bates et al), which was subsequently converted into TAG. The amount of PC bound delta-12-desaturase substrate and product that was part of the delta-12-desaturase reaction was therefore also directly linked to the substrate specificity of PDCT.

There was a strong correlation of the delta-12-desaturation conversion efficiency with the copy number of the delta-12-desaturase (Figure 32). This was evident when comparing the average conversion efficiency of T0 single copy plants (across all events per construct), with the average conversion efficiency of T0 double copy plants (across all events per

construct). The same correlation can be seen in the T1 generation. The strong correlation of the delta-12-desaturation conversion efficiency with the copy number of the delta-12-desaturase was also seen when comparing copy number differences due to heterozygous vs homozygous T-DNA integrations: The T0 generation always consist of heterozygous plants, whereas the T1 generation largely consists of homozygous plants due to the selection applied in Example 10 through Example 18. The delta-12-desaturation conversion efficiency increases when comparing the average conversion efficiency of T0 single copy plants (across all events per construct), with the average conversion efficiency of T1 single copy plants (across all events per construct). Similarly, the delta-12-desaturation conversion efficiency increases when comparing the average conversion efficiency of T0 double copy plants (across all events per construct), with the average conversion efficiency of T1 double copy plants (across all events per construct). The only exceptions are:

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM337-1qcz rc: while not as pronounced as for VC-LJB2197-1qcz and VC-LLM306-1qcz rc, there are also subtle differences here in delta-12-desaturase conversion efficiencies. The slight differences are due to insufficient data in the T0 generation to accurately assign the events to the two copy number categories for events containing the T-DNAs of construct VC-LJB2197-1qcz and VC-LLM337-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc: while not as pronounced as for VC-LJB2197-1qcz and VC-LLM306-1qcz rc, also here the subtle differences in delta-12-desaturase conversion efficiencies are due to insufficient data in the T0 generation to accurately assign the events to the two copy number categories for events containing the T-DNAs of construct VC-LJB2197-1qcz and VC-LLM338-3qcz rc

T1 single copy events vs T1 single copy (single locus) events containing the construct VC-LTM593-1qcz rc: , there was no effect on the conversion efficiency due to the 2-fold copy number difference between these groups. The group of single copy (single locus) events is affected by an additional phenomenon whereby the average VLC-PUFA level in T2 seeds of T1 plants of this homozygous group decreases instead of increasing compared to segregating T1 seeds of heterozygous T0 plants. A further indication that this group was an outlier group can be seen in Figure 35, where it was evident that the delta-5-desaturase

conversion efficiency was significantly lower in homozygous plants compared to heterozygous plants.

Considering the high conversion efficiency of the delta-12-desaturase, it is likely hat the provision of substrate via LPCAT is not a bottleneck. Also, the data suggest that efficient removal of delta-12-desaturase products contributes to the high conversion efficiency. However; taking into account the high levels of 18:2n-6 accumulated in the oil and the low delta-6-conversion efficiency, it was evident that not all delta-12-desaturated 18:2n-6 was removed from PC via trans-esterification to CoA (the site of action for the next pathway step). With respect to the fate of 18:2n-6; our observations indicate that the delta-12-desaturated 18:2n-6 was likely removed by the direct conversion of unsaturated PC into DAG via PDCT as discussed by Bates et al. (2014). This activity of PDCT strongly supports a high delta-12-desaturase conversion efficiency by providing fresh 18:1n-9-PC substrate to the delta-12-desaturase, while simultaneously removing 18:2n-6-PC product (by conversion into DAG). However, the latter activity poses a strong bottleneck for the continuation of the pathway, which is evident by the low delta-6-desaturase conversion efficiencies.

This effect of increased conversion efficiency of the delta-12-desaturase has been observed when analyzing the PUFA profile of more than 6.000 plants, that have been obtained by transforming more than 300 multi-gene constructs into Canloa and Arabidopsis, whereby all of these constructs caried genes having the essentail activities requiered for Arachidonic acid synthesis. Across these more then 300 constructs, more than 10 different delta-12-desaturase enzymes have been investigated.

Conversion efficiency of delta-6-desaturation

For the delta-6-desaturation, it was of particular importance to highlight that conversion efficiencies observed for this step are dependent on the substrate concentration (which was CoA bound linoleic acid), catalyst concentration (which was the delta-6-desaturase), and product concentration. It was, that PC bound 18:2n-6 produced by the delta-12-desaturase needs to be trans-esterified to CoA before delta-6-desaturation can take place. The absence of accumulation of 18:3n-6 and 18:4n-3 in the examples 10 -18 strongly indicates that CoA-bound 18:3n-6 and CoA-bound 18:4n-3 was efficiently converted by the delta-6-elongase, effectively preventing any significant accumulation of 18:3n-6 and 18:4n-3. In general, delta-6-desaturase conversion efficiencies are low for all constructs. Considering efficient removal of product was not a bottleneck, the low conversion efficiencies can be

either due to low activity of the delta-6-desaturase enzyme, or because of inefficient conversion of PC-bound substrate into CoA-bound substrate. Regardless of this, there was a clear correlation of the delta-6-desaturation conversion efficiency with the copy number of the delta-6-desaturase. In a manner similar to that of the delta-12-desaturase, this was seen regardless of the reason for the copy number increases (heterozygous vs homozygous, single copy genomic integrations vs double copy genomic integrations). In fact, plants of the construct combination VC-LJB2197-1qcz and VC-LLM306-1qcz rc contain an additional copy of the delta-6-desaturase on the T-DNA. It was therefore consistent that the group of 22 double copy T1 plants would have in the T2 seeds the highest delta-6-desaturase conversion efficiencies of all similar double copy groups of different constructs. For the delta-6-desaturase, the same exceptions to this copy number effect as for the delta-12-desaturase are seen. The same for the interpretation mentioned for the delta-12-desaturase of why these exceptions are observed are assumed to be the reasons for the delta-6-desaturase also applies in this case.

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM306-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM337-1qcz rc

T0 single copy events vs T0 double copy events containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc

T1 single copy events vs T1 single copy(single locus) events containing the construct VC-LTM593-1qcz rc

As demonstrated for the delta-12-desaturase, the amount of delta-12-desaturase product increases with increasing copy number of the delta-12-desaturases, which not only indicates an increased amount of delta-12-desaturase protein, but also implies an increased amount of substrate for the delta-6-desaturase. As both the delta-12-desaturases and the delta-6-desaturase are contained on the same T-DNA in all examples 10 to 18, the copy number of the delta-6-desatuases will also increase when copy number of the delta-12-desaturase increases, with the exceptions of events where truncated insertion of the T-DNA contain either the delta-12-desaturase and not the delta-6-desaturases, or vice versa.

In summary, not only was the effect of the gene copy duplication of the delta-12-desaturase surprising, but it was also unexpected that the increased copy number of delta-6-

desaturase led to an increased amount of delta-6-desaturase protein, and this increased amount of protein can convert the additional amount of delta-12-desaturated fatty acids with an increased conversion rate. To illustrate the effect, here as an example the comparison of two groups shown in examples 10 to 18 that have a gene copy number difference of "four":

All 275 heterozygous T0 plants containing a single copy of the T-DNA of construct VC-LTM593-1qcz rc

All 64 homozygous T1 plants containing two copies the T-DNA of of construct VC-LTM593-1qcz rc at two different chromosomal loci

In group A, the total sum of fatty acid that contain a double bond due to the activity of the delta-12-desaturase was 47.3% and total sum of fatty acids that contain a double bond due to the activity of the delta-6-desaturase was 11.2%. Consequently, 23.7% of all fatty acids that underwent delta-12-desaturation also underwent delta-6-desaturation.

In group B, the total sum of fatty acid that contain a double bond due to the activity of the delta-12-desaturase was 61 % and total sum of fatty acid that contain a double bond due to the activity of the delta-6-desaturase was 23.7%. Consequently, 38.8% of all fatty acids that underwent delta-12-desaturation also underwent delta-6-desaturation.

As a result of that, the sum of all fatty acids downstream of the delta-6-desaturase in the pathway more than doubled in group B versus group A.

This effect of increased conversion efficiency of the delta-6-desaturase has been observed when analyzing the PUFA profile of more than 6.000 plants, that have been obtained by transforming more than 300 multi-gene constructs into Canloa and Arabidopsis, whereby all of these constructs caried genes having the essentail activities requiered for Arachidonic acid synthesis. Across these more then 300 constructs, more than 5 different delta-6-desaturase enzymes have been investigated.

Conversion efficiency of delta-6-elongation

As was evident in Figure 34, the conversion efficiency of the delta-6-elongation was very high regardless of the copy number. Because of that, copy number mediated effects on the conversion efficiency are sometimes insignificant, but are sometimes clearly observed. E.g. the events obtained for the construct combinations VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc and for the combination LJB2755-2qcz rc and VC-LTM217-1qcz rc contain the

lowest delta-6-elongation conversion efficiencies of all constructs, which can be attributed to the fact that these construct combinations encode just one delta-6-elongation enzyme [c-d6Elo(Tp_GA2)], instead of two [(c-d6Elo(Tp_GA2), c-d6Elo(Pp_GA2)].

Conversion efficiency of delta-5-desaturation

The data in Figure 35 do not indicate a consistent construct independent copy number dependence of the delta-5-desaturase.

Conversion efficiency of omega-3-desaturation

Figure 36 shows the omega-3-desaturase conversion efficiencies that have been calculated by excluding the C18 substrates and products shown in Figure 2, thereby excluding the delta-15-desaturase specific activity of omega-3-desaturases. Figure 37 shows the omega-3-desaturase conversion efficiencies that have been calculated by including the C18 substrates and products, thereby including the delta-15-desaturase specific activity of omega-3-desaturases. As both omega-3-desaturases used in the invention have virtually no delta-15-desaturase activity, conclusions on the effect of copy number on the omega-3-desaturase are best drawn from Figure 36. While not as pronounced as observed for the delta-12-desaturase or delta-6-desaturase, there is, in many cases, a clear link between omega-3-desaturases copy number and the omega-3-desaturase conversion efficiency. This was particularly evident as plants of the construct combination as VC-LJB2197-1qcz + VC-LLM338-3qcz rc display the lowest omega-3-desaturase conversion efficiency of all other constructs, which can be attributed to the fact that this construct combination encodes just one omega-3-desaturase enzyme [c-o3Des(Pi_GA2)], instead of two [(c-o3Des(Pi_GA2), c-o3Des(Pir_GA)]. Exceptions to the copy number dependence of the omega-3-desaturase conversion efficiency are:

Plants containing the T-DNAs of construct RTP10690-1qcz_f

Plants containing the T-DNAs of construct VC-LTM595-1qcz rc

Plants containing the T-DNAs of VC-LJB2197-1qcz and VC-LLM338-3qcz rc

Conversion efficiency of delta-5-elongation

The complex interdependency of all the pathway step conversion efficiencies between each other can be seen by comparing Figure 38 with Figure 35: the high delta-5-elongation conversion efficiency for two groups of plants containing the T-DNAs of construct VC-LTM593-1qcz rc (813 T1 plants of the double copy group, 700 plants of the double

copy/single locus group) was in fact not due to a high delta-5-elongation conversion efficiency, but rather due to an inefficient supply of substrate to the delta-5-elogase at the delta-5-desaturase pathway step. Similar to the omega-3-desaturase, while not as pronounced as for the delta-12-desaturase or delta-6-desaturase, there was a link between delta-5-elongase copy number and the delta-5-elongase conversion efficiency.

Conversion efficiency of delta-4-desaturation

As can be seen in Figure 39, there was no direct evidence for an increase in delta-4-desaturase conversion efficiency to delta-4-desaturase copy number. The highest delta-4-desaturase conversion efficiency has been observed for plants containing the T-DNAs of construct LJB2755-2qcz rc and VC-LTM217-1qcz rc. This construct combination differs only from the combination VC-LJB2755-2qcz rc + VC-LLM391-2qcz rc in replacing the d4Des(Eg) by the c-d4Des(PI_GA)2. As a conclusion of the plant data shown in Example 10 to 18 and the yeast data shown in Example 22, it can be clearly seen that the delta-4-desaturation conversion efficiency reaches an upper limit using the genes c-d4Des(Tc_GA) and d4Des(Eg), that cannot be overcome by increased gene copy numbers. All constructs that encode the c-d4Des(PI_GA)2 instead of d4Des(Eg) have a higher delta-4-desaturation conversion efficiency, and the only exception to that are plants containing the construct RTP10690-1qcz_F. As shown in Example 25, this was due to very low expression of both delta-4-desaturases. This in turn highlights that the conversion efficiency does depend on the gene expression, but cannot overcome an upper limit.

Example 20: Environmental effects on VLC-PUFA levels. Correlation between oil content, VLC-PUFA levels observed conversion efficiencies at each pathway step

Analysing the VLC-PUFA levels presented in the Examples 10 to 18, we have oberserved that there is, in most cases, a level of variability, within a genetically identical population of plants (e.g. a genetically stable event where all plants are genetically identical), even when these plants are grown in a controlled greenhouse environment. To illustrate the effect more clearly, VLC-PUFA profiles of seed batches having highest EPA+DHA levels among several type of plant populations are shown in the examples 10 to 18. Such plant populations are (1) either genetically identical due to the T-DNA integrations (i.e. all plants of the same event, where either all or at least most are homozygous for all T-DNA integrations in those events), or (2) identical related to the T-DNA copy number, and these populations have been grown in the same environment. For example, out of 249 segregating T1 seedlings,

10 T1 plants for event LBFDAU (see Example 18) have been identified in the greenhouse to be identical in having one complete T-DNA integration, and one truncated T-DNA integration, whereby both T-DNA insertions where found to be homozygous (Table 135). In a field trial conducted in parallel, out of about segregating T1 seedlings, 4 plants have been identified as being homozygous for both T-DNA insertions (Table 144). The average EPA+DHA content measured in randomly selected seed from seed batches of these 10 greenhouse grown plants was found to be 13.9% EPA + 2.6% DHA. Similarly, the EPA+DHA content of the 4 field grown plants was found to be 13.4% EPA + 2.7% DHA. Among the 10 greenhouse grown plants was a plant that produced up to 15.1% EPA + 3.0% DHA (Table 139). Similarly, among the 4 field grown plants was a plant that produced up to 17.6% EPA + 3.6% DHA (Table 146). Analysing single seed of this field grown plant, single seeds having up to 26.2% EPA + 4.9% DHA, or 23.6%EPA + 5.8% DHA where found (Table 147). The lowest EPA+DHA content found among these 95 seed contained 13.5% EPA and 2.5% DHA, and the average content among these seed was 18.2% EPA and 3.7% DHA, which matched the content measured in the random selection of twice 15 seed as shown in Table 146 (17.6% EPA + 3.6% DHA). This event LBFDAU is just a representative example to illustrate for that every event and all constructs exhibit plant-to-plant variability, and seed-to-seed variability. The extent of the plant to plant variability is shown in the examples 10 to 18, where it is shown that the average PUFA profile of a certain population (e.g. all plants belong to the same event and are genetically identical, or all plants are single copy, heterozygous) is always different than the profile observed in individual plants. To ilustrate the magnitude of this variability, examples 10 to 18 show the average PUFA profile of such populations and also the profile of the single plant having the highest EPA+DHA levels.

In conclusion, while the event LBFDAU was capable of producing in a genetically identical plant population ~13.5% EPA + 2.6% DHA in a given environment, there are individual plants within this population that produce up to ~18% EPA + 3.7% DHA as an average of all seeds produced by this plant, and single seed of this plant can reach up to ~26% EPA and ~5% DHA. These differences in EPA+DHA levels are due to the interaction of the genetic constituents of the event with the environment. However, it was consistently observed that comparable populations yield higher VLC-PUFA levels in the greenhouse compared to the field. This trend usually correlates with lower oil content in the greenhouse, compared to the field. To investigate this observation in more detail, oil content measured in all homozygous seed batches of the single copy event LANPMZ (event described in example 11) was

plotted in Figure 40 against the sum of all VLC-PUFA that are downstream of the delta-12-desaturase (see Figure 2) measured in the same seed batches. The same has been done for event LAODDN (event described in example 13), and is plotted Figure 41. This analysis was alos done for two events described in example 18, namely for event LBFGKN (Figure 42), and for event LBFLFK (Figure 43). What was observed is a strong correlation between these two parameters. The same analysis for wildtypes (Figure 72) reveals no such correlation, instead, Figure 72 shows a difference between greehouse grown and field grown wildtype plants, in that field grown wildtype plants have higher levels of 18:2n-6 and 18:3n-3 and lower 18:1n-9 compared to greenhouse grown wildype plants.

To analyse in detail if this correlation depicted for event LANPMZ in Figure 40 can be attributed to certain pathway steps, conversion efficiencies for each pathway step and for each seedbatch analysed in Figure 40 have been calculated, and plotted in Figure 44, Figure 48, Figure 52, Figure 56, Figure 60, Figure 64, and Figure 68. The same analysis has been done for event LAODDN, and is plotted in Figure 45, Figure 49, Figure 53, Figure 57, Figure 61, Figure 65 and Figure 69. The same analysis was also been done for event LBFGKN, (plotted in Figure 46, Figure 50, Figure 54, Figure 58, Figure 62, Figure 66 and Figure 70), and for event LBFLFK (Figure 47, Figure 51, Figure 55, Figure 59, Figure 63, Figure 67 and Figure 71). Comparing all these figures, one can see that the correlation observed in Figure 40 and Figure 41 is largely attributed to the delta-12-desaturase pathway step (Figure 44 to Figure 47), the delta-6-desaturase pathway step (Figure 48 to Figure 51), and the delta-4-desaturase pathway step (Figure 68 to Figure 71): in these figures, there is a construct and event independent negative correlation between the pathway steps and the seed oil content visible, which cannot be observed for the other pathway steps.

The negative correlation of the delta-12-desaturase pathway with seed oil content in event LANPMZ was observed in wild type plants, as showin in Figure 72. Moreover, Figure 72 shows that field grown wildtype plants have higher levels of 18:2n-6 and 18:3n-3 and lower 18:1n-9 compared to greenhouse grown wildype plants. This can most likely be explained with previous observations (Xiao et al. 2014), in that the native Canola endogenous delta-12-desaturase was regulated by temperature and that temperature regulation of delta-12-desaturase at the transcript level and protein level is a recurring them in plants (Kargiotidou et al. 2008, Tang et al. 2005, Sanchez-Garcia et al. 2004) for the regulation of this enzyme, whereby under field conditions the delta-12-desaturase be would up-regulated compared to

greenhouse conditions. However, it can be concluded that there was no regulation of the delta-12-desaturase in dependance of the oil content observed in wildtype canola plant. Consequently comparing Figure 72 with Figure 40 to Figure 43), it can furthermore be concluded that only the introduced transgeneic delta-12-desaturase was subject to a regulation that was seed oil content dependant.

A further obersvation is that field grown transgenic plants do not have a higher delta-12-desaturase conversion efficiency compared to greenhouse grown plants, even though this was clearly oberved for wildtype plants in Figure 72. It can be concluded that the endogenous native delta-12-desaturase does not significantly contribute to the overall observed delta-12-desaturase conversion efficiency in the transgenic plants of the invention.

In addition to plotting pathway step conversion efficiencies vs seed oil content, levels of some key fatty acids have been plotted agains seed oil content (Figure 81, Figure 82, Figure 83, Figure 84). The data have been fitted in those figures by using all datapoints of all depicted events to determine the slope of the regression, and by subsequently using all datapoints for single events to determine the intercept of the regression line with the x-axis (0% oil content) of the regressions shown in (Figure 81, Figure 82, Figure 83, Figure 84). The results of this analysis are shown in Table 158.

Table 158: Regressions equations of fitting the data in Figure 81, Figure 82, Figure 83, Figure 84 as described in example 20. Listed are the parameters for the equation (Fatty acid) = (seed oil content) * Slope + Intercept.

Event		EPA+DHA vs oil		ARA vs oil		EPA vs oil		DHA vs oil	
	N	Intercept	Slope	Intercept	Slope	Intercept	Slope	Intercept	Slope
LBFDAU	23	28.054	-0.398	5.774	-0.098	22.053	-0.288	6.003	-0.110
LBFPRA	44	26.075	-0.398	7.399	-0.098	20.762	-0.288	5.315	-0.110
LBFLFK	42	25.280	-0.398	5.788	-0.098	19.578	-0.288	5.703	-0.110
LBFDHG	9	25.058	-0.398	5.235	-0.098	19.167	-0.288	5.892	-0.110
LANBCH	60	24.910	-0.398	7.142	-0.098	19.305	-0.288	5.606	-0.110
LBFGKN	291	23.481	-0.398	5.856	-0.098	17.994	-0.288	5.488	-0.110
LBFIHE	47	22.841	-0.398	6.086	-0.098	17.489	-0.288	5.353	-0.110
LBFDGG	44	22.800	-0.398	5.789	-0.098	17.403	-0.288	5.398	-0.110

Example 21: In vitro demonstration of enzyme activity

Desaturase Enzyme Activity in Microsomes Isolated from Transgenic Yeast

Expression of desaturases and elongases was accomplished in *Saccharomyces cerevisiae*. Briefly, yeast strains containing the appropriate plasmid were grown overnight at 30 °C (in SD-medium-uracil + raffinose) and then used to inoculate a larger culture at a starting OD_{600}

= 0.2 (in SD-medium-uracil+raffinose+galactose). After 24 hours at 30 °C the culture (typically OD_{600} = 0.6-0.8) was harvested by centrifugation and washed once in 25 mM Tris Buffer (pH 7.6). Preparation of crude extracts and microsomes from yeast expressing genes encoding desaturases and elongases was accomplished using standard procedures. Briefly, cells expressing desaturases were resuspended in 2 ml Desaturase Disruption Buffer (0.1M potassium phosphate pH 7.2, 0.33 M sucrose, 4 mM NADH, 1 mg/ml BSA (fatty acid free), 4000 U/ml catalase and protease inhibitors (Complete EDTA-free (Roche)) and disrupted using silica/zirconium beads in a BeadBeater. The crude extract was clarified by centrifugation twice at 8,000xg, 4 °C). After an additional centrifugation at 100,000xg (30 minutes at 4 °C) the microsomes were pelleted and ultimately resuspended in Desaturase Disruption Buffer (300 microliters). Protein concentrations in both the crude extract and microsomes were measured using the bicinchoninic acid (BCA) procedure (Smith, P. K., et al (1985) *Anal. Biochem.* (150): 76–85).

General Desaturase Activity Assays:
In the desaturase assay a [^{14}C]-labeled acyl-CoA was provided as a substrate and after the reaction the acyl-CoAs (and phospholipids) were hydrolyzed and methylated to fatty acid methyl esters (FAMEs), which were analyzed using argentation-TLC. The general assay conditions were modified from Banas et al. (Banas et al. (1997) *Physiology, Biochemistry and Molecular Biology of Plant Lipids* (Williams, J.P., Khan, M.U. and Lem, N.W. eds.) pp. 57-59).

The assay contained: 1 mg enzyme (crude extract) or 150 µg (microsomal fraction), 10 nmol [^{14}C]-acyl-CoA (3000 dpm/nmol), 7.2 mM NADH (total), 0.36 mg BSA (total) in a buffer comprised of 0.1 M K-phosphate pH 7.2, 0.33 M sucrose, 4 mM NADH, 1 mg/ml BSA and protease inhibitors in a total volume of 200 µl. After incubation at 30 °C for the desired time, 200 µl of 2 M KOH in MeOH:H_2O (1:4) was added and incubated for 20 minutes at 90 °C. Fatty acids were extracted by addition of 3 M HCl (200 µl), 1.5 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (500 µl). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:Acetic Acid (70:30:1) as a solvent. The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Delta-12 desaturase (*Phytophthora sojae*), c-d12Des(Ps_GA) Enzyme Activity: Enzyme assays were performed using re-suspended microsomes isolated from a yeast strain expressing the c-d12Des(Ps_GA) protein and compared to microsomes isolated from a control yeast strain containing an empty vector (LJB2126). In the presence of [^{14}C]18:1n-9-CoA, 16:0-lysphosphatidylcholine (LPC), and NADH membranes containing c-d12Des(Ps_GA) form an [^{14}C]18:2-fatty acid that can be isolated as a methyl ester and resolves on $AgNO_3$-TLC [heptane:diethyl ether (90:10)] similar to known synthetic standards. This enzyme activity requires NADH and was not observed in membranes isolated from the empty vector control strain. Control assays without 16:0-LPC contain a small-amount of activity, presumably due to endogenous 16:0-LPC found in yeast microsomes. Furthermore, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolabled fatty acid methyl esters demonstrated that all of the c-d12Des(Ps_GA) enzymatically produced 18:2n-6-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction. c-d12Des(Ps_GA) enzyme activity may also be demonstrated using other [^{14}C]acyl-CoA's which may include, but are not limited to: [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA.

Delta-6 desaturase (*Ostreococcus tauri*), c-d6Des(Ot_febit) Enzyme Activity: c-d6Des(Ot_febit) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Des(Ot_febit) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using $AgNO_3$-TLC and Heptane:Diethyl ether:Acetic Acid (70:30:1) as a solvent. Furthermore, the c-d6Des(Ot_febit) enzyme can be shown to directly desaturate an acyl-CoA substrate, as described in section C, as suggested in previous reports (Domergue et al. (2005) Biochem. J. 389: 483-490).

Delta-5 desaturase (*Thraustochytrium* ssp.), c-d5Des(Tc_GA2) Enzyme Activity: c-d5Des(Tc_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d5Des(Tc_GA2) protein using an [^{14}C]acyl-CoA general assay as described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA,

[^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Omega-3 desaturase (*Phytophthora infestans*), c-o3Des(Pi_GA2) Enzyme Activity: c-o3Des(Pi_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-o3Des(Pi_GA2) protein using [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Omega-3 desaturase (*Pythium irregulare*), c-o3Des(Pir_GA) Enzyme Activity: c-o3Des(Pir_GA) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-o3Des(Pir_GA) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Thraustochytrium* ssp.), c-d4Des(Tc_GA) Enzyme Activity: c-d4Des(Tc_GA) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Tc_GA) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Pavlova lutheri*), c-d4Des(Pl_GA)2 Enzyme Activity: c-d4Des(Pl_GA)2 enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Pl GA)2 protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to:

[^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-.18) and acetonitrile (100%) as a solvent.

Delta-4 desaturase (*Euglena gracilis*), c-d4Des(Eg) Enzyme Activity: c-d4Des(Eg) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d4Des(Eg) protein using an [^{14}C]acyl-CoA in the general assay described above. [^{14}C]Acyl-CoA's may include, but are not limited to: [^{14}C]18:1n-9-CoA, [^{14}C]18:2n-6-CoA, [^{14}C]20:3n-6-CoA, [^{14}C]20:4n-6-CoA, [^{14}C]22:5n-3-CoA. Isolated fatty acid methyl esters derived from enzymatic substrates and products can be resolved using Reverse Phase-TLC (Silica gel 60 RP-18) and acetonitrile (100%) as a solvent.

Desaturase Activity in Microsomes isolated from Transgenic *Brassica napus*.

Microsomes containing recombinant desaturases and elongases capable of synthesizing docosahexaenoic acid (22:6n-3) were isolated from immature seeds from transgenic *B. napus* using a procedure adopted from Bafor, M. et al. Biochem J. (1991) 280, 507-514. Briefly, immature seeds were first seperated from canola pods and then the developing embryos were isolated from the seed coat and transferred to ice-cold 0.1M Phosphate buffer (pH 7.2). The developing embryos were then washed with fresh Phosphate buffer, transferred to an ice-cold mortar, and ground to a homogenous solution in Extraction Buffer (0.1 M Phosphate, pH 7.2, 0.33 M sucrose, 1 mg/ml BSA (essentially fatty acid free), 4000U/ml catalase, 4 mM NADH and protease inhibitor-Complete EDTA-free (Roche)). The lysed developing embryo's were diluted 20-fold with additional Extraction Buffer and passed through 2 layers of Miracloth into a centrifuge tube. Following centrifugation at 18,000 x g for 10 minutes at 4 °C, the clarified supernatant was passed through Miracloth into an ultracentrifuge tube. Following centrifugation at 105, 000 xg for 60 minutes at 4 °C, the supernatant was removed from the microsomal pellet, which was then washed once with Extraction Buffer, and then using a Dounce homogenizer resuspended as a homogenous solution in Extraction Buffer (about 1 ml per 500 embryo's).

Enzyme activity can be demonstrated for the desaturases using the assays described above in Section A. for microsomes isolated from yeast expression strains.

In summary in Section A we have provided a method that allows unambiguous demonstration of fatty acyl desaturase enzyme activity. We provide data demonstrating that: (1) gene c-d12Des(Ps_GA) encodes a delta-12 desaturase protein from *Phytophthora sojae* (c-d12Des(Ps_GA) that desaturates oleic acid (18:1n-9) to form linoleic acid (18:2n-6) in both microsomes isolated from a transgenic yeast (Figure 24, panel A) and from a transgenic *B. napus* event (Figure 25, panel A) expressing this protein, (2) gene c-o3Des(Pi_GA2) encodes a protein from *Phytophthora infestans* (c-o3Des(Pi_GA2)) that desaturates arachidonic acid (20:4n-6) to form eicosapentaenoic acid (20:5n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel B) expressing this protein, (3) gene c-o3Des(Pir_GA) encodes an omega-3 desaturase protein from *Pythium irregulare* (c-o3Des(Pir_GA)) that desaturates arachidonic acid (20:4n-6) to form eicosapentaenoic acid (20:5n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel B) expressing this protein, (4) gene c-d4Des(Tc_GA) encodes a delta-4 desaturase protein from *Thraustochytrium* sp. (c-d4Des(Tc_GA)) that desaturates docosapentaenoic acid (22:5n-3) to form docosahexaenoic acid (22:6n-3) in microsomes isolated from a transgenic yeast (Figure 24, panel C) expressing this protein, (5) a transgenic *B. napus* event containing both the gene encoding the delta-4 desaturase protein from *Thraustochytrium* sp, gene c-d4Des(Tc_GA), and the gene c-d4-Des(Pl_GA) from *Pavlova Lutheri* contains at least one enzyme, localized to the microsomes, capable of desaturating docosapentaenoic acid (22:5n-3) to form docosahexaenoic acid (22:6n-3) (Figure 25, panel C). Except for the c-d12Des(Ps_GA), which has a known endogenous enzyme in Brassica, all other examples presented contain no detectable endogenous desaturase activity in microsomes isolated from either control yeast strains (Figure 24) or control *Brassica* lines (Figure 25).

Using the methods described in Section A for desaturase proteins the level of expression or detected enzyme activity may be influenced by the presence or absence of fusion tags to the native protein. Fusion tags or proteins to the desaturases may be attached the amino-terminus (N-terminal fusions) or the carboxy-terminus (C-terminal fusions) of the protein and may include but are not limited to: FLAG, hexa-Histidine, Maltose Binding Protein, and Chitin Binding Protein.

We have provided methods to establish enzyme catalyzed desaturation reactions required in an engineered pathway to biosynthesize docosohexaenoic acid (DHA, 22:6n-3) from oleic acid (18:1n-9) in canola. The methods presented in Example 21, Section A were developed to demonstrate desaturase activity in yeast strains expressing individual

desaturases and can be further used to confirm the respective desaturase enzyme activities in transgenic canola, as described and demonstrated in Example 21, Section B. Furthermore these methods can be incorporated, by one skilled in the art, to measure desaturase enzyme activities in other organisms including, but not limited to: *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Brassica* spp., *Camelina sativa*, *Carthamus tinctorius*, and *Salvia hispanica*.

Desaturase Headgroup (CoA vs PC) Preference

Fatty acid desaturases catalyze the abstraction of two hydrogen atoms from the hydrocarbon chain of a fatty acid to form a double bond in an unsaturated fatty acid and can be classified according to the backbone that their substrate was connected to: an acyl-CoA, an acyl-ACP (ACP, acyl carrier protein) or an acyl-lipid. To date a few examples exist where the acyl-CoA substrate has been confirmed. These involve purified enzymes and examples include a Linoleoyl-CoA Desaturase (Okayasu et al. (1981) Arch. Biochem. Biophys. 206: 21-28), a stearoyl-CoA desaturase from rat liver (Strittmatter et al (1974) Proc. Nat. Acad. Sci. USA 71: 4565-4569), and a Stearoyl-ACP desaturase from avocado (Shanklin J and Somerville C (1991) Proc Natl Acad Sci USA 88:2510-2514).

Alternatively, Heinz and coworkers have reported a strategy employing *in vivo* feeding of substrates to yeast strains expressing desaturases to examine substrate specificity of desaturases (Domergue et al. (2003) J. Biol. Chem. 278: 35115-35126 , Domergue et al. (2005) Biochem. J. 389: 483-490). In these studies predictions of a desaturases's preference for acyl-lipid substrates were based on data obtained from a thorough analysis of the desaturated products in the acyl-CoA, phospholipid and neutral lipid pools over a growth time course. However, highly active acyltransferases which transfer acyl-groups between various pools (e.g. CoA, ACP, and lipid) may influence or convolute these data (Domergue et al. (2005) Biochem. J. 389: 483-490, Meesapyodsuk, D., Qui, X. (2012) Lipids 47: 227-237). Therefore this approach was still limited by the absence of direct evidence, such as obtained from *in vitro* assays, needed for conclusive determination of the substrate form of the desatrurase.

Herein, we provide a previously unreported method to distinguish between enzymes that desaturate acyl-CoA fatty acids from enzymes that desaturate phospholipid linked fatty acids using microsomal preparations of proteins. We have improved upon initial reports of similar strategies to generate [^{14}C]-phosphatidylcholine analogs *in situ* (Stymne, S., and

Stobart, A. K. (1986) Biochem. J. 240: 385-393, Griffiths, G., Stobart, A. K., and Stymne, S. (1988) Biochem. J. 252: 641-647) by: (1) monitoring the initial acyl-transfer reaction catalyzed by LPCAT to establish that all of the [^{14}C]-acyl-CoA has been consumed, and (2) including exogenous lysophosphatidyl choline (LPC). Our improvements therefore establish that only [^{14}C]-phosphatidylcholine analogs are present upon initiation of the desaturase assay and allow for testing of other phospholipids by adding their corresponding lysolipid.

Demonstration of Phosphatidylcholine Specificity:

To test if a desaturase accepts an acyl-lipid (e.g. a phospholipid) substrate the enzyme reaction was performed as described above (Section A, General Desaturase Activity Assays), but after a pre-incubation and in the presence and absence of exogenous lysophosphatidyl choline (LPC). The microsomal fraction of the yeast strain expressing the enzyme of interest was pre-incubated with a [^{14}C]-labelled acyl-CoA substrate in the presence of 16:0-lysophosphatidyl choline, which was typically 50 μM but may vary from 0 – 500 μM. During the pre-incubation endogenous lysophosphatidyl choline acyl transferase (LPCAT), present in the microsomes, transfers the [^{14}C]fatty acid from CoA to 16:0-LPC generating, *in situ*, a [^{14}C]fatty acid-phosphatidylcholine (PC) (Jain et al. (2007) *J. Biol. Chem.* 282:30562-30569, Riekhof et al. (2007) *J. Biol. Chem.* 282:36853-36861, Tamaki et al. (2007) *J. Biol. Chem.* 282:34288-34298)). After a pre-incubation (typically 15 minutes, but may vary from 1 – 300 minutes) essentially all of the [^{14}C]-labelled acyl-CoA substrate was consumed, as measured by scintillation counting and TLC of the aqueous phase. Control reactions are performed in the absence of exogenous 16:0-LPC.

The reaction was stopped and lipids were extracted using the method of Bligh and Dyer (Bligh, E. G., and Dyer, J. J. (1959) *Can J. Biochem. Physiol.* 37: 911-918), by addition of 200 μl 0.15 M acetic acid and 1 ml MeOH:$CHCl_3$ (1:1). Part (about 10%) of the $CHCl_3$ phase (containing phosphatidyl choline (PC) and free fatty acids (FFA's)) was analyzed by scintillation counting and the rest was applied to a silica thin layer chromatography (TLC) plate. The plate was first developed in a polar solvent [$CHCl_3$:MeOH:acetic acid (90:15:10:3) and then in Heptane:diethylether:acetic acid (70:30:1) to measure incorporation into PC and the amount of FFA's (likely generated by thioesterases). PC and FFA's were scraped off the the plate and methylated by addition of MeOH containing 2% H_2SO_4 at 90 °C for 30 minutes. The methyl esters were extracted in

hexane and analyzed as as described above for the respective enzymes (Example 21, Section A). The upper (aqueous) phase of the reaction mixture extraction contains acyl-CoA's and was hydrolyzed by addition of an equal volume of 2 M KOH in MeOH:H_2O (1:4) and incubated for 20 minutes at 90 °C. Part of the aqueous phase was then analyzed by scintillation counting before fatty acids were extracted by addition of 3 M HCl (1 ml), 3.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (1 ml). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:acetic acid (70:30:1) as a solvent or Reverse Phase-TLC (Silica gel 60 RP-18 using acetonitrile (100%)). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Delta-12 desaturase (*Phytophthora sojae*), c-d12Des(Ps_GA) Substrate Preference:
The c-d12Des(Ps_GA) enzyme activity demonstrated in Section A can be further characterized to establish the backbone of the oleic acid substrate. In the desaturase assay described in Section C containing 16:0-lysphosphatidylcholine (LPC) substantial desaturation was observed. A significantly reduced, but detectable, desaturase activity was observed in control reactions lacking 16:0-LPC which likely results from acylation of endogenous LPC present in the yeast microsomes containing the d12Des(Ps_GA) protein. Additionally, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolable fatty acid methyl esters demonstrated that all of the d12Des(Ps_GA) enzymatically produced 18:2n-6-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction (Figure 26, Panel A).

Delta-4 desaturase (*Thraustochytrium* ssp.), c-d4Des(Tc_GA) Substrate Preference:
The c-d4Des(Tc_GA) enzyme activity demonstrated in Section A can be further characterized to establish the backbone of the docosopentaenoic acid substrate. In the desaturase assay described in Section C without additional 16:0-lysphosphatidylcholine (LPC), desaturation was observed (Figure 26, Panel B), and likely results from the presence of endogenous 16:0-LPC present in the membranes containing the c-d4Des(Tc_GA) protein. The c-d4Des(Tc_GA) desaturase activity was dramatically stimulated by including additional 16:0-LPC in the assay (Figure 26, Panel B),

consistent with the observation endogenous lysophosphatidyl choline acyl transferase (LPCAT), present in the microsomes, transfers the [^{14}C]22:5n-3 from CoA to 16:0-LPC generating a [^{14}C]22:5n-3-phosphatidylcholine (PC) that was desaturated. Additionally, separation of the phospholipids from the free-fatty acids after the enzymatic reaction and characterization of the isolable fatty acid methyl esters demonstrated that essentially all of the c-d4Des(Tc_GA) enzymatically produced 22:6n-3-fatty acid methyl ester (FAME) was found in the phosphatidylcholine fraction (Figure 26, Panel B and Panel C).

Demonstration of acyl-CoA specificity:

The assay conditions were as described above in Section A (General Desaturase Activity Assays). Microsomal protein from yeast expressing d9Des(Sc) was used and to the assay, up to 5 nmol of 20:1-CoA was added. This assay may also include alternative acyl-CoA's such as: 18:1n-9-CoA, 18:2n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 22:5n-3-CoA. The reaction was stopped and lipids were extracted using the method of Bligh and Dyer (Bligh, E. G., and Dyer, J. J. (1959) *Can J. Biochem. Physiol.* **37**, 911-918), by addition of 200 µl 0.15 M acetic acid and 1 ml MeOH:$CHCl_3$ (1:1). Part (about 10%) of the $CHCl_3$ phase (containing phosphatidyl choline (PC) and free fatty acids (FFA's)) was analyzed by scintillation counting and the rest was applied to a silica thin layer chromatography (TLC) plate. The plate was first developed in a polar solvent [$CHCl_3$:MeOH:acetic acid (90:15:10:3) and then in Heptane:diethylether:acetic acid (70:30:1) to measure incorporation into PC and the amount of FFA's (likely generated by thioesterases). PC and FFA's were scraped off the plate and methylated by addition of MeOH containing 2% H_2SO_4 at 90 °C for 30 minutes. The methyl esters were extracted in hexane and analyzed as described above for the respective enzymes (SectionA). The upper (aqueous) phase of the reaction mixture extraction contains acyl-CoA's and was hydrolyzed by addition of an equal volume of 2 M KOH in MeOH:H_2O (1:4) and incubated for 20 minutes at 90 °C. Fatty acids were extracted by addition of 3 M HCl (1 ml), 3.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (1 ml). The chloroform phase was recovered, dried under N_2(g) and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by $AgNO_3$-TLC and Heptane:Diethyl ether:acetic acid (70:30:1) as a solvent or Reverse Phase-TLC (Silica

gel 60 RP-18 using acetonitrile (100%)). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.
To demonstrate acyl-CoA dependency both methods are tested. If desaturation does not occur in the method for determining PC-specificity (LPC addition and preincubation before adding NADH) and the method for determining acyl-CoA specificity (20:1-CoA addition) leads to the desaturated product in the H_2O-phase, it can be concluded that the enzyme was acyl-CoA dependent (see Figure 27).

In summary, we presented a previously unreported method to distinguish between enzymes that desaturate acyl-CoA fatty acids from enzymes that desaturate phospholipid linked fatty acids. This embodment of the invention t uses microsomal preparations of enzymes and does not, as in previous examples, require purification of the enzyme of interest. Furthermore, this embodiment allows isolation of the intact desaturated enzymatic product, allowing characterization of the backbone to which it was linked (e.g. lipid-, CoA-, or free fatty acid). An important consideration was that the endogenous lysophosphatidyl choline acyl transferase (LPCAT) present in yeast-derived microsomes can utilize a broad range of acyl-CoA's (Jain et al. (2007) *J. Biol. Chem.* 282:30562-30569, Riekhof et al. (2007) *J. Biol. Chem.* 282:36853-36861, Tamaki et al. (2007) *J. Biol. Chem.* 282:34288-34298)) making it suitable for generating an extensive variety of different phosphatidylcholine derivatives for assaying desaturase enzymes. LPCAT is able to accept 18:1n-9-CoA and 20:4n-6-CoA and this enzyme can acylate LPC with 22:5n-3-CoA.. Microsomes isolated from any cells or tissue can be used in this embodiment of the invention, including but not limited to bacterial cells (e.g. *Escherichia coli*, *Psuedomonas aeruginose*, *Bacillus thuringiensis*), mammalian tissue (e.g. liver) and plant tissue (e.g. leafs, roots, seeds, and pods) and could use exogenously supplied lysophosphatidyl choline acyl transferase from *Saccharomyces cerevisiae*, if necessary. Slight modifications to the general method presented here may include a pre-incubation with alternate acyl-CoA's, not the potential desaturase substrate, which could reduce the observed background due to endogenous LPC present in the membranes and also minimize thioesterase degradation of enzyme substrate or product acyl-coA's.

Elongase Activity.

Expression of elongase enzymes in yeast was performed as described above for the desaturase enzymes in Section A. Isolation of microsomes containing expressed elongases was generally as described above in Section 1 and by Denic (Denic, V. and Weissman (2007) Cell 130, 663-677). Briefly, cells from a yeast expression culture (50 ml) were resuspended in 1 ml of Elongase Disruption Buffer (20 mM Tris-HCl, pH 7.9, 10 mM $MgCl_2$, 1 mM EDTA, 5% glycerol, 0.3 M ammonium sulfate, protease inhibitor), mixed with 1 ml silica/zirconium beads (0.5mm) and disrupted in a BeadBeater. After centrifugation (two times for 5 minutes at 8000xg, 4 °C) the crude extract was recovered and after a second centrifugation (100,000xg, 2 hours at 4 °C), the microsomal fraction was resuspended in 500 µl of assay buffer (50 mM HEPES-KOH pH 6.8, 150 mM KOAc, 2 mM MgOAc, 1 mM $CaCl_2$, protease inhibitor). The protein concentrations in the microsomes were measured according to the BCA method. Resuspended microsomes were aliquoted and frozen in N_2(l) and stored at -80 °C.

In the elongase assay [^{14}C]-labeled malonyl-CoA and non-labeled acyl-CoA were provided as substrates. After the reaction has proceeded an appropriate time, which may vary between 0 - 300 minutes depending on the purpose of the experiment, the reaction mixture was subjected to hydrolysis and methylation and the FAMEs were analyzed by RP-TLC combined with by electronic autoradiography using Instant Imager.

The assay contains about 170 µg microsomal protein, 7.5 nmol [^{14}C]malonyl-CoA (3000 dpm/nmol), 5 nmol acyl-CoA in a total volume of 100 µl. After incubation for the desired time at 30 °C, the reaction was stopped with the addition of 100 µl of 2 M KOH in MeOH (1:4) followed by a 20 minute incubation at 90 °C. Fatty acids were extracted by addition of 3 M HCl (100 µl), 0.75 ml of MeOH:$CHCl_3$ (2:1) and $CHCl_3$ (250 µl). The chloroform phase was recovered, dried under N_2(g), and fatty acids were methylated by addition of 2 ml MeOH containing 2% H_2SO_4 and incubation of 30 minutes at 90 °C. FAMEs were extracted by addition of 2 ml H_2O and 2 ml hexane and separated by Reverse Phase-TLC (Silica gel 60 RP-18) using a solvent of acetonitrile:tetrahydrofuran (85:15). The radioactive lipids were visualized and quantified by electronic autoradiography using Instant Imager.

Furthermore, assays may include additional components (e.g. 1 mM NADPH, 2 mM $MgCl_2$, and 100 μM cerulenin) to complete the fatty acid reduction cycle by endogenous yeast enzymes, but limit further elongation of the acyl- CoA.

Delta-6 Elongase (*Thalassiosira pseudonana*), c-d6Elo(Tp_GA2) Enzyme Activity: c-d6Elo(Tp_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Elo(Tp_GA2) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

Delta-6 Elongase (*Physcomitrella patens*), c-d6Elo(Pp_GA2) Enzyme Activity: c-d6Elo(Pp_GA2) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d6Elo(Tp_GA2) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

Delta-5 Elongase (*Ostreococcus tauri*), c-d5Elo(Ot_GA3) Enzyme Activity: c-d5Elo(Ot_GA3) enzyme activity and substrate specificity can be demonstrated in microsomes isolated from a yeast strain expressing the c-d5Elo(Ot_GA3) protein using [^{14}C]malonyl-CoA and an acyl-CoA in the general elongase assay described above. Acyl-CoA's may include, but are not limited to: 18:1n-9-CoA, 18:2n-6-CoA, 18:3n-6-CoA, 20:3n-6-CoA, 20:4n-6-CoA, 20:5n-3-CoA, 22:5n-3-CoA.

In the presence of NADPH and [^{14}C]malonyl-CoA, 18:3n-6-CoA was elongated to 20:3 n-6-CoA by the delta-6 Elongases isolated from *Thalassiosira pseudonana* (Tp) and *Physcomitrella patens* (Pp) as shown if Figure 28, panels A and B. In both delta-6 elongase reactions the observed FAME-product co-migrates with 20:3n-6-methyl ester standards and was radioactive, consistent with transfer of two-carbons from [^{14}C]-malonyl-CoA to 18:3n-6-CoA. In the presence of NADPH the fatty acid reduction cycle was completed resulting in a saturated enzymatic product. However in the absence of NADPH a derivative of the direct enzymatic product, 3-keto-20:3n-6-CoA, was isolated

as a FAME. The isolated enzymatic product was decarboxylated and converted to the 2-keto-19:3n-6-FAME as described previously (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol. Chem. 281: 9018-9029). Appropriate controls demonstrate that this elongation reaction was dependent upon either the Delta-6 Elo (Tp) or the Delta-6 Elo (Pp) and not catalyzed by endogenous yeast enzymes.

In the presence of NADPH and [^{14}C]malonyl-CoA , 20:5n-3-CoA was elongated to 22:5 n-3-CoA by the c-d5Elo(Ot_GA3), and containing either an N-terminal FLAG tag or a C-terminal FLAG tag, as shown in Figure 28, panel C. In the Delta-5 elongase reaction the observed FAME-product co-migrates with a 22:5n-3-methyl ester standard and was radioactive, consistent with transfer of two-carbons from [^{14}C]-malonyl CoA to 20:5n-3-CoA. In the presence of NADPH the fatty acid reduction cycle was completed resulting in a saturated enzymatic product. However in the absence of NADPH a derivative of the direct 3-keto-22:5n-3-CoA product was isolated as a FAME. The isolated enzymatic product was decarboxylated and a 2-keto-21:5n-3-FAME as described previously (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol. Chem. 281: 9018-9029). Appropriate controls demonstrate that this elongation reaction was dependent upon the Delta-5 Elo (Ot) and not catalyzed by endogenous yeast enzymes.

Herein, using a highly sensitive elongase assay, we have demonstrated the enzyme activities of the Delta-6 Elongases used (Figure 28, panel A and B) and a Delta-5 Elongase (Figure 28, panel C), enzymes that are central to engineering canola to biosynthesize docosahexaenoic acid. For each of these elongases we have shown that in the presence of [^{14}C]malonyl-CoA and the appropriate fatty-acyl CoA ester substrate these enzymes can transfer two-carbons (containing [^{14}C]) from malonyl-CoA to the appropriate fatty-acyl-CoA ester to synthesize a new fatty acid which has been elongated by two carbons. In some cases a derivative (decarboxylated 2-keto compound) of the direct enzymatic product (3-Keto-acylCoA ester) of the elongase was observed, however in the absence of NADPH only this decarboxylated 2-keto compound was observed, consistent with previous observations by Napier (Bernert, J.T and Sprecher, H. (1977) J. Biol. Chem. 252:6736-6744 and Paul et al (2006) J. Biol.

Chem. 281: 9018-9029).

In summary we have provided a method that allows unequivocal demonstration of fatty acyl elongation enzyme activity. We provide data demonstrating that: (1) gene c-d6Elo(Tp_GA2) encodes a delta-6 elongase protein from *Thalassiosira pseudonana* (c-d6Elo(Tp_GA2)) that converts 18:3n-6-CoA to 20:3n-6-CoA in microsomes isolated from a transgenic yeast (Figure 28, panel A), (2) gene c-d6Elo(Pp_GA2) encodes a delta-6 elongase protein from *Physcomitrella patens* (c-d6Elo(Pp_GA2)) that converts 18:3n-6-CoA to 20:3n-6-CoA in microsomes isolated from a transgenic yeast (Figure 28, panel B), (3) a transgenic *B. napus* event containing both the gene encoding for the delta-6 elongase protein from *Thalassiosira pseudonana*, gene c-d6Elo(Tp_GA2), and the gene encoding for the gene the delta-6 elongase protein from *Physcomitrella patens*, gene c-d6Elo(Pp_GA2), contains at least one enzyme, localized to the microsomes, capable of elongating 18:3n-6-CoA to 20:3n-6-CoA (Figure 28, panel A) (4) gene c-d5-Elo(Ot_GA3) encodes a delta-5 elongase protein from *Ostreococcus tauri* (c-d5Elo(Ot_GA3)) that converts 20:5n-3-CoA to 22:5n-3-CoA in microsomes isolated from both a transgenic yeast (Figure 28, Panel C) and transgenic *B. napus* event (Figure 29, Panel B). In all examples presented no endogenous elongase activity was detected in microsomes isolated from either control yeast strains (Figure 28) or control Brassica lines (Figure 29).

Using the methods described in Section D for elongase proteins the level of expression or detected enzyme activity may be influenced by the presence or absence of fusion tags to the native protein. Fusion tags or proteins to the desaturases may be attached the amino-terminus (N-terminal fusions) or the carboxy-terminus (C-terminal fusions) of the protein and may include but are not limited to: FLAG, hexa-Histidine, Maltose Binding Protein, and Chitin Binding Protein.

We have provided methods to establish enzyme catalyzed elongase reactions required in an engineered pathway to biosynthesize docosohexaenoic acid (DHA, 22:6n-3) from oleic acid (18:1n-9) in canola. The methods presented in Example 21 were developed to demonstrate elongase activity in yeast strains expressing individual elongases and can be further used to confirm the respective elongase enzyme activities in transgenic

canola. Furthermore these methods can be incorporated, by one skilled in the art, to establish elongase enzyme activities in other organisms including, but not limited to: *Saccharomyces cerevisiae*, *Arabidopsis thaliana*, *Brassica* spp., *Camelina sativa*, *Carthamus tinctorius*, and *Salvia hispanica*.

Example 22: In vivo demonstration of mode of action: substrate specificity, substrate selectivity

Cloning of Genes into Yeast Expression Vectors:

For single gene expression, the yeast expression vector pYES2.1/V5-His-TOPO (Invitrogen) was used. Flanking primers were designed according to the manufacturer's instructions, and genes were amplified from plant expression vectors using the proof-reading polymerase Phusion high-fidelity polymerase (New England Biolabs). After agarose gel electrophoresis PCR fragments were cut out and purified using an EZ-10 spin column gel extraction kit (Bio Basic Inc.), cloned into the yeast expression vector pYES2.1/V5-His-TOPO (Invitrogen), transformed into *E. coli*, and gene orientation was checked by PCR. For co-expression of multiple genes, genes were cloned into pESC yeast expression vectors (Stratagene) under the control of the GAL1 promotor. To do this, appropriate restriction sites were introduced upstream and downstream of coding regions via PCR, followed by fragment isolation, TA-cloning into the pGEM-T vector, release of the gene fragment by enzyme digestion, and ligation into the pESC vector. For all constructs plasmids were isolated using an EZ-10 spin column plasmid DNA miniprep kit (Bio Basic Inc.). All constructs were sequenced prior to yeast transformation. After sequencing, plasmids were transformed into Saccharomyces cerevisiae (yeast) strain INVSc1 (Invitrogen), using the Sc EasyComp Transformation Kit (Invitrogen) according to the manufacturer's protocol, and selected on plates lacking the appropriate amino acids.

Expression of Heterologous Genes in Yeast:

Yeast cultures were grown overnight at 30°C in drop out base (DOB-URA: 1.7 g/L yeast nitrogen base, 5 g ammonium sulfate, and complete supplement mixture minus appropriate amino acids for selection) containing 2 % glucose. The OD600 of the overnight yeast cultures were obtained and culture concentrations were standardized between samples. The samples were washed with DOB-URA containing 2 % galactose

and expression was carried out for 3 days at 20 °C in the same media supplemented with exogenous fatty acids and 0.01 % tergitol. For exogenous fatty acid feeding, cells were fed with 0.25 mM of the appropriate fatty acids, except where indicated otherwise. Fatty acid substrates and FAME standards were purchased from Nu-Chek Prep Inc (Elysian, MN).

Fatty Acid Analysis by Gas Chromatography:

5 mL cultures were precipitated by centrifugation and washed once with induction buffer and once with water. The supernatant was removed, and 2 mL of 3N methanolic-HCl (Supelco) was added to the cell pellet. After gentle mixing, the mixture was incubated at 80°C for 40 min, cooled to room temperature, and 1 mL 0.9% NaCl plus 2 mL hexane was added. The sample was vortexed and centrifuged, and the hexane phase was removed and dried under nitrogen gas. Fatty acid methyl esters (FAMEs) were resuspended in 100 µl hexane and analyzed by gas chromatography using an Agilent 6890N gas chromatograph equipped with a DB-23 column. The thermal program used was 160 °C for 1 min, then temperature was increased to 240 °C at a rate of 4 °C/min. FAMEs were identified based on known standards and the conversion percent was calculated as: [(Product)x100%]/(Substrate + Product).

Time Course Studies:

Samples were grown overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C. For some time course studies, samples were pre-induced overnight prior to feeding as indicated in results. Samples (1 ml of culture) were collected at the indicated time intervals, washed once with 0.5% tergitol and once with double distilled water, and pellets were stored at -80°C until the completion of the time course. Fatty acid extraction and GC analysis basically followed section 3, however 1 mL methanolic HCl and 0.5 mL 0.9% NaCl and 1mL hexane were used for fatty acid extraction, and the final resuspension was performed in 100 µL hexane.

Feeding Studies with Individual Fatty Acids:

Samples were grown overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C for 3 days. Measurements from at least 3 clones were used to calculate averages.

Feeding Studies with Multiple Fatty Acids:

Fatty acids for co-feeding were selected based on results from experiments described above where cells expressing each construct were fed with all possible substrates. The positive substrates for a given enzyme were fed together, however, if one substrate formed a product that also was a known substrate (for example, with certain elongases), separate mixtures were used. For omega-3-desaturases, two mixtures, each containing SDA as a standard, were used for feeding. Initially, cultures were fed mixtures containing 0.25 mM total fatty acid substrate. However, due to differences in uptake, it was necessary to optimize fatty acid mixtures so that the levels of Substrate A + Product A was equal to Substrate B + Product B within ± 5%. The final fatty acid mixtures used were also subjected to GC to obtain an estimation of relative uptake. After appropriate fatty acid mixtures were obtained, induction and feeding was performed as described above (Expression of Heterologous Genes in Yeast). After induction, yeast cells were induced for 3 days at 20 °C before harvesting of cells and GC analysis as described above (Expression of Heterologous Genes in Yeast and Fatty Acid Analysis by Gas Chromatography). All experiments were replicated three times.

Co-expression Heterologous Genes in Yeast:

Protocols for co-expression of genes basically followed those provided for single gene expression experiments. Cultures were grow overnight at 30°C, sample concentrations were standardized prior to feeding, and cultures were induced at 20°C for 3 days. The following sets of genes were co-expressed (pY = pYES2.1/V5-His-TOPO ; pT= pESC-Typ, pL=pESC-Leu):

1) pY-c-d12Des(Ps_GA) /pT-c-d6Des(Pir_GA)
2) pY-c-d12Des(Ps_GA)/pT-c-d6Des(Ot_febit)
3) pY-c-d6Elo(Tp_GA2)/pT-c-d6Des(Ot_febit)
4) pY-c-d6Elo(Tp_GA2)/pT-c-d6Des(Pir_GA)
5) pL- c-d6Elo(Tp_GA2)/pY-c-d5Des(Tc_GA2)
6) pL-c-d8Des(Eg) /pY-c-d5Des(Tc_GA2)
7) pL- c-d6Elo(Tp_GA2)/pY-c-d5Des(Sa)

8) pL-c-d8Des(Eg)/pY-c-d5Des(Sa)

Isolation and Analysis of Lipids:

For expression of desaturases or elongases, yeast cultures were grown overnight at 30°C in drop out base (DOB-ura) containing 2% glucose. The samples were washed with induction media (DOB-ura) containing 2% galactose and expression was induced for 3 days at 20 °C in the same media supplemented with the appropriate fatty acids and 0.01 % tergitol (NP-40). After 3 days of incubation, yeast cells were collected by centrifugation and washed twice with distilled water. Fifteen mL of chloroform:methanol (1:1) was added to the collected yeast pellet and the sample was incubated at room temperature with shaking for 3 hr. After the 3 hr incubation, the sample was centrifuged and the aqueous phase was collected and stored at -20°C. Chloroform:methanol (2:1) was added to the pellet, and it was incubated overnight at 4°C, then subjected to centrifugation. The aqueous phase was collected and pooled with the aqueous phase collected previously. Nine mL of 0.45% NaCl was added to the pooled aqueous phase, the sample was vortexed and the organic phase was collected after separated by centrifugation at 1500 x g for 3 min. The organic phase was dried under nitrogen gas and resuspended in 100 uL of chloroform to give the total fatty acid fraction. 5 uL of the total fatty acid fraction was analyzed by GC, and the remainder was separated by TLC as described below.

TLC plates were heat activated for at least 3 hrs at 120°C before use. The mobile phase used to separate the fatty acids consisted of chloroform : methanol : acetic acid (65:35:8) and primuline (5mg in 100mL acetone : water, 80:20) was used to visualize the separated lipid fractions under UV light. The individual lipid fractions (PC, PE, PI+PS, and neutral lipids) were identified using standards and removed separately from the TLC plate. To extract the lipids from the silica, 400 uL water, 2 mL chloroform and 2 mL methanol were added, the sample was vortexed vigorously, and 2 mL of 0.2 M H_3PO_4/ 1M KCl was added. The lower organic phase was collected and the remaining aqueous phase was re-extracted by adding 2 mL of chloroform. The pooled organic phase was dry under nitrogen gas, and the fatty acid profile of individual lipid fractions was analyzed by gas chromatography. GC was performed by adding 2 mL of 3N methanolic HCl, followed by incubation at 80 °C for 40 min, then adding 1 ml of 0.9% NaCl and 2 mL of hexane and vortexing. The hexane phase was collected, dried under nitrogen gas, and resuspended in 100 µL of hexane for GC analysis. Gas

chromatography was performed using an Agilent 6890N gas chromatograph equipped with a DB-23 column and a thermal program consisting of: 160 °C for 1 min, then temperature was increased to 240 °C at a rate of 4 °C/min. After the initial GC analysis, samples with low concentrations were re-suspended in 40µL of hexane and re-analyzed.

Time Course studies:

Initially, a range of conditions were tested with the c-d5Des(Tc_GA2) (Figure 30). When cultures were fed with 0.25 mM exogenous substrate at the beginning of induction the amounts of product and substrate and the desaturation percentage remained steady from approximately 48-92 hours (Figure 30, Panel A). Therefore, these conditions were used for further time course experiments.

To determine if these conditions were acceptable for all gene constructs, time courses were conducted with yeast cultures expressing all 10 genes individually. The preferred substrate for each enzyme was supplied exogenously (Figure 31).

Although the rate of uptake of fatty acids and the activity of enzymes varied, the levels of product, substrate and percent elongation or desaturation was relatively stable at the 72 hour time point (Figure 31). Therefore data collection at 72 hours was used in all further experiments, except where noted otherwise.

Analysis of all enzymes with all fatty acids:

The major activity of each desaturase and elongase used in the plant construct was determined upon gene isolation. However, many desaturases and elongases have secondary activities. When all enzymes are present together, such secondary activities affect the overall production rates of desired fatty acids, or lead to the production of side-products. In this experiment, we tested all enzymes with all fatty acids that might be expected to be present in a plant expressing all 10 genes. The enzymes and fatty acids used in this experiment are listed below:

Enzyme	Fatty acid
c-d12Des(Ps_GA)	18:1n-9
c-d6Des(Ot_febit)	LA (18:2n-6)
c-d6Elo(Pp_GA2)	ALA (18:3n-3)
c-d6Elo(Tp_GA2)	GLA (18:3n-6)
c-d5Des(Tc_GA2)	SDA (18:4n-3)
c-o3Des(Pi_GA2)	DHGLA (20:3n-6)
c-o3Des(Pir_GA)	ARA (20:4n-6)
c-d5Elo(Ot_GA3)	DTA (22:4n-6)
c-d4Des(Pl_GA)2	DPAn-3 (22:5n-3)
c-d4Des(Tc_GA)	DPAn-6 (22:5n-6)
	DHAn-3 (22:6n-3)

Although each individual substrate fatty acid was tested with each individual enzyme, results are only given for fatty acid/enzyme combinations where a detectable level of activity was obtained (see Table 159, Table 160, and Table 161).

Although we saw some unexpected activities with the desaturases, such as the conversion of GLA to SDA by the c-d5Des(Tc_GA2) and conversion of DHGLA to ARA by the c-d4Des(Tc_GA), these activity levels were low and produce fatty acids that would be expected in the DHA synthesis pathway (Table 159).

Table 159. Conversion percentages of exogenous fatty acids by desaturases.

Enzyme	Substrate	Product	% desaturation
c-o3Des(Pir_GA)	GLA	SDA	2.7 ± 0.05
	DPAn-6	DHAn-6	6.93 ± 0.85
	ARA	EPA	45.44 ± 2.40
	LA	ALA	6.29 ± 0.28
	DTA	DPAn-3	25.57 ± 1.16
	DHGLA	ETA	31.64 ± 3.38
c-o3Des(Pi_GA2)	GLA	SDA	7.84 ± 0.53
	DPAn-6	DHAn-3	4.77 ± 0.78
	ARA	EPA	40.0 ± 3.38

Enzyme	Substrate	Product	% desaturation
	LA	ALA	5.47 ± 0.76
	DTA	DPAn-3	17.86 ± 0.43
	DHGLA	ETA	39.71 ± 2.37
c-d5Des(Tc_GA2)	DHGLA	ARA	58.03 ± 2.01
	GLA	SDA	0.23 ± 006
c-d4Des(Tc_GA)	DPAn-3	DHAn-3	20.74 ± 1.43
	DTA	DPAn-6	17.33 ± 0.24
	DHGLA	ARA	1.15 ± 0.12
c-d4Des(Pl_GA)2	DPAn-3	DHAn-3	43.78 ± 3.85
	DTA	DPAn-6	44.26 ± 4.83
c-d12Des(Ps_GA)	18:1	LA	57.04 ± 0.49
	16:1*	16:2	32.42 ± 0.64
c-d6Des(Ot_febit)	LA	GLA	68.82 ± 1.89
	ALA	SDA	71.0 ± 0.68

The elongases generally showed a higher level of side activities (Table 160, Table 161). While the two delta-6 elongases showed similar conversion percentages for the main substrates GLA and SDA, the P. patens enzyme showed higher levels of activity on LA and ALA, and only the P. patens delta-6 elongases showed detectable activity of ARA and EPA. Nonetheless, activity levels were substantially higher with GLA and SDA, suggesting that if these fatty acids, which were expected to be available in the pathway, would be the preferred substrate of the enzyme.

Table 160. Conversion percentages of exogenous fatty acids by Delta-6 Elongases

Enzyme	Substrate	Product	% Elongation
c-d6Elo(Pp_GA2)	GLA	DHGLA	80.23 ± 0.81
	SDA	20:4n-3	85.67 ± 1.18

Enzyme	Substrate	Product	% Elongation
	LA	20:2n-6	20.86 ± 1.23
	ALA	20:3n-3	31.19 ± 0.23
	ARA	DTA	2.42 ± 0.08
	EPA	DPAn-3	5.16 ± 0.29
c-d6Elo(Tp_GA2)	GLA	DHGLA	70.11 ± 1.37
	SDA	20:4n-3	85.55 ± 0.32
	LA	20:2n-6	2.22 ± 0.15
	ALA	20:3n-3	4.63 ± 0.35

The c-d5Elo(Ot_GA3) was capable of consecutive elongations of most fatty acids (Table 161). However, the highest conversion by a large margin was for the desired EPA to DPAn-3 conversion. In the engineered plant system, the two delta-4 desaturases are available to desaturate DPAn-3, preventing further elongation. The levels of fatty acids containing 24 carbons produced by this elongase are small given that high levels of substrate are present in the yeast system and no competing enzymes are present.

Table 161. Conversion percentages of exogenous fatty acids by c-d5Elo(Ot_GA3) elongase. Substrate and Product values represent % of total fatty acids.

Substrate	Product 1	Product 2	Product 3	% Elongation
LA	**20:2n-6**			
24.34 ± 1.28	0.41 ± 0.07			1.64 ± 0.18
DHGLA	**22:3n-6**			
12.92 ± 0.93	1.24 ± 0.22			8.73 ± 1.10
DHAn-3	**24:6n-3**			
9.41 ± 0.86	1.37 ± 0.12			12.84 ± 1.84
DPAn-3	**24:5n-3**			
10.85 ± 0.46	2.15 ± 0.28			16.59 ± 2.37
GLA	**DHGLA**	**22:3n-6**		
23.95 ± 3.22	1.0 ± 0.13	0.24 ± 0.03		4.91 ± 0.24
ARA	**22:4n-6**	**24:4n-6**		
7.12 ± 1.33	11.23 ± 0.28	0.26 ± 0.05		61.92 ± 5.22
EPA	**DPAn-3**	24:5n-3		

Substrate	Product 1	Product 2	Product 3	% Elongation
0.85 ± 0.01	6.76 ± 0.85	1.74 ± 0.21		90.8 ± 0.98
SDA	**20:4n-3**	**22:4n-3**	**24:4n-3**	
14.42 ± 1.18	0.47 ± 0.04	4.17 ± 0.12	0.26 ± 0.03	25.48 ± 1.72
ALA	**20:3n-3**	**22:3n-3**	**24:3n-3**	
20.09 ± 3.23	1.79 ± 0.28	1.70 ± 0.35	0.15 ± 0.03	15.38 ± 2.06

Since 20:4n-3 was not commercially available was generated in vivo by co-expression of c-d6Elo(Tp_GA2) with each of the other desaturases or elongases. Therefore upon supplying exogenous SDA, 20:4n-3 was produced by the c-d6Elo(Tp_GA2) and the efficiency of conversion of this fatty acid by the second enzyme was measured.

Table 162. Enzyme activity with 20:4n-3.

Helper Enzyme (delta-6 elongase) pL vector	2nd enzyme in pY vector	delta-6 elongase conversion efficiency (%)	Conversion efficiency of 2nd enzyme (%)	Gene dosage effect
c-d6Elo(Tp_GA2)	c-d4Des(Pl_GA)2	70.0 ± 1.2	N/A	N/A
c-d6Elo(Tp_GA2)	c-d4Des(Tc_GA)	69.1 ± 0.7	3.3 ± 0.2	N/A
c-d6Elo(Tp_GA2)	c-d5Des(Tc_GA2)	64.3 ± 1.4	86.2 ± 1.0	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	72.9 ± 2.1	2.9 ± 0.2	N/A
c-d6Elo(Tp_GA2)	c-d12Des(Ps_GA)	70.3 ± 1.3	N/A	N/A
c-d6Elo(Tp_GA2)	c-o3Des(Pi_GA2)	67.4 ± 3.6	N/A	N/A
c-d6Elo(Tp_GA2)	c-o3Des(Pir_GA)	70.0 ± 1.1	N/A	N/A
c-d6Elo(Tp_GA2)	c-d5Elo(Ot_GA3)	68.8 ± 1.4	80.2 ± 2.5	N/A
c-d6Elo(Tp_GA2)	c-d6Elo(Pp_GA2)	89.8 ± 0.7	N/A	YES (Δ6 ELO)
c-d6Elo(Tp_GA2)	c-d6Elo(Tp_GA2)	90.3 ± 0.8	N/A	YES (Δ6 ELO)

* Cultures were fed with SDA and 20:4n-3 (delta-6 elongation) was produced as a substrate for either desaturases or elongases. Three repeats were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector. All genes were placed under the control of the Gal1 promoter. N/A: not applicable; DES: desaturase; ELO: elongase

The c-d4Des(Tc_GA), the c-d5Des(Tc_GA2), the c-d6Des(Ot_febit), and the c-d5Elo(Ot_GA3) were able to use 20:4n-3 as a substrate (Table 162). The c-d5Des(Tc_GA2) showed the highest conversion percentage, which would be expected as 20:4n-3 was a known substrate for delta-5 desaturases. The c-d5Des(Tc_GA2) was also able to produce 22:4n-3. When using two delta-6 elongases in one construct, a gene dosage effect was observed; the delta-6 elongase activity increased in the presence of two c-d6Elo(Tp_GA2), or a gene pair consisting of a c-d6Elo(Tp_GA2) and a c-d6Elo(Pp_GA2).

Fatty acid specificity of desaturases and elongases of this invention

Enzymes were provided with mixtures of fatty acids to allow comparisons of relative activity when two substrates were present concurrently; an enzyme may be more active on substrate A than substrate B when they are supplied individually, but when they are supplied at the same time the relative activity of the enzyme on each substrate could change. Fatty acids of the same length were preferably provided at the same time, since they would be most likely to be present in the plant concurrently. Mixtures of fatty acids were adjusted so that the level of (substrate 1 + product 1) = (substrate 2 + product 2) within ± 5%.

Table 163. Fatty acid preferences of elongases and desaturases

Gene (Fatty Acid Mix)	Substrate	% conversion
c-d5Elo(Ot_GA3)	EPA	90.36 ± 2.17
(EPA/ARA/DHGLA)	ARA	40.96 ± 4.50
	DHGLA	3.14 ± 0.47
c-d5Elo(Ot_GA3)	DPAn-3	4.75 ± 0.57
(DPAn-3/DHA)	DHA	5.69 ± 0.56
c-d5Elo(Ot_GA3)	LA	0.57 ± 0.06
(LA/ALA)	ALA	13.43 ± 1.65
c-d5Elo(Ot_GA3)	GLA	2.89 ± 0.06
(GLA/SDA)	SDA	29.24 ± 0.73
c-d4-Des(Tc_GA)	DTA	21.01 ± 2.28
(DTA/DPAn-3)	DPAn-3	25.18 ± 2.08
c-d4-Des(Pl_GA)2	DTA	34.41 ± 1.63
(DTA/DPAn-3)		
	DPAn-3	41.27 ± 2.07
c-d6-Des(Ot_febit)	LA	56.62 ± 2.06

Gene (Fatty Acid Mix)	Substrate	% conversion
(LA/ALA)	ALA	77.66 ± 0.80
c-d6-Elo(Pp_GA2)	LA	11.38 ± 0.94
(LA/ALA)	ALA	24.41 ± 1.84
c-d6-Elo(Pp_GA2)	GLA	81.59 ± 1.66
(GLA/SDA)	SDA	85.23 ± 1.28
c-d6-Elo(Tp_GA2)	LA	1.93 ± 0.15
(LA/ALA)	ALA	6.41 ± 0.68
c-d6-Elo(Tp_GA2)	GLA	81.61 ± 1.03
(GLA/SDA)	SDA	87.70 ± 0.87
c-d5-Des(Tc_GA2)	DHGLA	85.0 ± 2.38
(DHGLA/ETA)	ETA (20:4n-3)	90.75 ± 1.15
c-o3-Des(Pi_GA2)	DHGLA	28.82 ± 1.26
(DHGLA/ARA/DTA)	ARA	39.15 ± 1.19
	DTA	7.32 ± 0.63
c-o3Des(Pir_GA)	DHGLA	25.22 ± 1.70
(DHGLA/ARA/DTA)	ARA	53.19 ± 2.52
	DTA	12.66 ± 1.17

As described in Table 163 the relative activities of the omega-3 desaturases follow the same order as described earlier (Table 159), but that the activity of both omega-3 desaturases was lower on DTA when DHGLA and ARA were available. Both delta-4 desaturases showed a preference for the omega-3 substrate when the omega-3 and omega-6 substrates were present in equal amounts. The preference of the c-d6Des(Ot_febit) for the omega-3 substrate was also increased when both substrates are available (Table 163). For the delta-5 elongase, the relative elongation of secondary substrates generally dropped when the primary, or preferred substrate was available (Table 163).

Additive enzyme activity supporting MoA (CoA vs PC)

The delta-5 desaturase genes from *S. arctica* and *Thraustochytrium* sp. were cloned into the pYES2.1/V5-His-TOPO expression vector, and both the c-d6Elo(Tp_GA2) and the c-d8Des(Eg) were cloned into the pESC-Leu expression vector. Yeast was transformed with the appropriate vector pairs and selection on DOB-uracil-leucine.

Positive cultures were grown and induced and GC analysis was conducted as described above in Example 23. All cultures were grown concurrently.

Table 164. Deduced mode of action of the Delta-5 desaturases.

Helper Enyzme known to work in PC (delta-8 DES)	delta-5 DES	delta-% DES Conversion efficiency	Deduced MoA of delta-5 DES
c-d8Des(Eg)	c-d5Des(Sa)	38 %	
c-d8Des(Eg)	c-d5Des(Tc_GA2)	36 %	
Helper Enyzme known to work in CoA (delta-6 ELO)	**delta-5 DES**	**delta-% DES Conversion efficiency**	
c-d6Elo(Tp_GA2)	c-d5Des(Sa)	32 %	PC
c-d6Elo(Tp_GA2)	c-d5Des(Tc_GA2)	80 %	CoA

As described in Table 164, the delta-5 desaturase activity of the c-d5Des(Sa) on HGLA was similar regardless if the DHGLA was derived from elongation in the coenzyme A (CoA) pool or desaturation in the phosphatidylcholine (PC) pool. Conversely, Table 164 also shows that the delta-5 desaturase activity of the c-d5Des(Tc_GA2) was much higher if the DHGLA substrate was derived from elongation in the CoA pool. In combination with data from lipid analysis by TLC, this suggests that the c-d5Des(Tc_GA2) was capable of desaturating substrates in the CoA pool, although it does not indicate that desaturation was limited to this pool.

The level of delta-6-elongation following desaturation by a desaturase active in the PC pool (c-d6Des(Pir_GA)) and a desaturase thought to be active in the CoA pool (c-d6Des(Ot_febit)) was determined. Since elongation takes place in the acyl-CoA pool, more efficient elongation can occur following desaturation by an acyl-CoA dependant desaturase. The two desaturases were also expressed in the presence of a delta -12 desaturase that was active on PC substrates, to allow the efficiency of subsequent delta -6 desaturation to be compared. Finally, the effects of individual desaturase genes and pairs of desaturase genes on elongase and desaturase activity were compared to determine dosage effects.

Table 165. Effects of various delta-6 desaturases on subsequent delta-6 elongation*.

Construct	delta- 6 Des %	delta- 6 ELO %
pT-c-d6Des(Ot_febit) & pY-c-d6Elo(Tp_GA2)	44.9 ± 1.0	89.3 ± 1.4
pT-c-d6Des(Pir_GA) & pY-c-d6Elo(Tp_GA2)	19.7 ± 0.5	63.7 ± 4.8

* Cultures were fed with LA and production of GLA (delta-6 desaturation) and HGLA (delta-6 elongation) were measured. pY: pYES2.1/V5-His-TOPO expression vector pT: pESC-trp expression vector. All genes were under the control of the Gal1 promoter.

Table 166. Effects of delta-12 desaturation on subsequent delta-6 elongation*.

Construct	delta-12 DES %	delta-6 DES %
pT-c-d6Des(Ot_febit) & pY-c-d12Des(Ps_GA)	60.2 ± 2.9	23.2 ± 0.1
pT-c-d6Des(Pir_GA) & pY-c-d12Des(Ps_GA)	62.8 ± 1.4	17.1 ± 1.7

* Cultures were supplied with exogenous 18:1n-9. The production of LA (delta-12 desaturation) and GLA (delta-6 desaturation) were measured. pY: pYES2.1/V5-His-TOPO expression vector pT: pESC-trp expression vector. All genes were under the control of the Gal1 promoter.

GLA conversion by the c-d6Elo(Tp_GA2) was higher with GLA derived from desaturation by the c-d6Des(Ot_febit) compared to GLA derived by desaturation with the c-d6Des(Pir_GA) (Table 165). This shows that c-d6Des(Ot_febit) acts in the acyl-CoA pool, making the resulting substrate more readily available for the delta-6 elongase, and therefore resulting in a higher elongase activity. Correspondingly, the delta-6 desaturase activity of the c-d6Des(Ot_febit) was reduced by almost half if the substrate was derived from delta-12 desaturation in the PC pool (Table 165 and Table 166), whereas the the delta-6 desaturase activity of the c-d6Des(Pir_GA) was similar whether the substrate (LA) was derived from delta-12-desaturation of OA in the PC pool, or was supplied exogenously (Table 165 and Table 166). Exogenously supplied substrate was believed to enter the yeast cell in the form of acyl-CoA. In combination with data from lipid analysis by TLC, this data suggests that the c-d6Des(Ot_febit) was acyl-CoA-dependent enzyme, consistent with a previous prediction (Domergue et al. (2005) Biochem. J. 389: 483-490).

Table 167. Effect of substrate pool and gene dosage on delta-6-desaturation and subsequent delta-6-elongation of ALA.

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	pT vector	64.3 ± 1.5	73.3 ± 0.2	N/A	CoA	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	pT vector	26.8 ± 11.0	53.0 ± 8.8	N/A	PC	N/A
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Ot_febit)	60.8 ± 2.3	80.8 ± 0.6	N/A	N/A	CoA

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Pir_GA)	27.9 ± 0.9	52.0 ± 0.9	N/A	N/A	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Pir_GA)	72.5 ± 2.2	71.1 ± 2.1	Yes (DES)	CoA	PC
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	c-d6Des(Pir_GA)	40.5 ± 1.3	47.5 ± 2.0	Yes (DES)	PC	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Ot_febit)	72.4 ± 0.9	78.1 ± 0.7	Yes (DES)	CoA	CoA

* Cultures were fed with ALA and production of SDA (delta-6 desaturation) and 20:4n-3 (delta-6 elongation) was measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All regulated by Gal1 promoter. N/A: not applicable; DES: desaturation conversion percent. Data from at least 3 clones were used for each measurement.

Table 168. Effect of substrate pool and gene dosage on delta-6-desaturation and subsequent delta-6-elongation of LA.

Helper Enzyme (delta-6 elongase) pL vector	First delta-6 desaturase pY vector	Second delta-6 desaturase pT vector	delta-6 desaturase conversion efficiency	delta-6 elongase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-6 desaturase	Deduced MoA of 2nd delta-6 desaturase
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	pT vector	60.3 ± 7.0	65.6 ± 1.8	N/A	CoA	N/A
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	pT vector	23.4 ± 15.5	43.2 ± 8.6	N/A	PC	N/A
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Ot_febit)	48.5 ± 0.2	75.6 ± 1.5	N/A	N/A	CoA
c-d6Elo(Tp_GA2)	pY vector	c-d6Des(Pir_GA)	23.0 ± 0.8	36.9 ± 2.2	N/A	N/A	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Pir_GA)	3.0 ± 1.4	60.1 ± 1.9	Yes (DES)	CoA	PC
c-d6Elo(Tp_GA2)	c-d6Des(Pir_GA)	c-d6Des(Pir_GA)	35.1 ± 2.2	35.4 ± 4.8	Yes (DES)	PC	PC
c-d6Elo(Tp_GA2)	c-d6Des(Ot_febit)	c-d6Des(Ot_febit)	62.5 ± 1.4	66.8 ± 2.5	Yes (DES)	CoA	CoA

* Cultures were fed with LA and production of GLA (delta-6 desaturation) and DHGLA (delta-6 elongation), three repeats were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All genes were placed under the control of the Gal1 promoter. N/A: not applicable; DES: desaturation conversion percent.

When co-expressing the c-d6Elo(Tp_GA2) and the c-d6Des(Ot_febit) (CoA), a higher elongase conversion efficiency was achieved compared to co-expressing the c-d6Elo(Tp_GA2) and the c-d6Des(Pir_GA) (PC), regardless of whether n-3 or omega-6 substrate was supplied exogenously (Table 167, Table 168). This was observed whether the relevant desaturase gene was cloned into the pYes vector or the pESC-trp vector. A slight increase in desaturation efficiency was observed in the presence of two

desaturase genes, particularly in cultures supplied with the omega-3 substrate ALA. Compared to cultures carrying a single c-d6Des(Ot_febit), the proportion of substrate elongated was only slightly enhanced in the presence of two acyl-CoA dependant c-d6Des(Ot_febit), or in the presence of both desaturases. Co-expression of the c-d6Elo(Tp_GA2) with two copies of c-d6Des(Pir_GA) (PC) resulted in the lowest elongation conversion level among the cultures carrying two desaturases. The presence of the with acyl-CoA dependent delta-6 desaturase contributed to a higher delta-6 elongation activity whether it was expressed alone or along with a second desaturase, further indicating the usefulness of this gene for VLC-PUFA synthesis.

The effect of substrate pool for various delta-4 desaturases was determined in the presence of a helper enzyme, the c-d5Elo(Ot_GA3), which was known to work in the CoA pool. The gene dosage effect of delta-4 desaturase genes was also determined.

Table 169. Effect of substrate pool and gene dosage effect for delta-4 desaturases (exogenous EPA supplied).

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	pT vector	29.9 ± 4.0	N/A	CoA	NA
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	pT vector	31.2 ± 2.8	N/A	PC	NA
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	c-d4Des(Tc_GA)	53.6 ± 1.0	++ (0.88)	CoA	PC
c-d5Elo(Ot_GA3)	c-d4Des(Eg)	c-d4Des(Tc_GA)	36.9 ± 3.5	Yes	PC	PC
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	c-d4Des(Tc_GA)	40.2 ± 0.2	+ (0.64)	PC	PC

* Cultures were fed with EPA and production of DPA (delta-5 elongation) and DHA (delta-4 desaturation) was measured for at least three samples. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-trp. All genes were placed under the control of the Gal1 promoter. N/A: not applicable. Dosage effect: number in brackets = conversion efficiency of two-desaturase construct/ (conversion efficiency of single gene A construct + conversion efficiency of single gene B construct).

Table 170. Effect of substrate pool and gene dosage effect for delta-4 desaturases (exogenous ARA supplied).

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase

Helper Enzyme (delta-5 elongase) pL vector	First delta-4 desaturase pY vector	Second delta-4 desaturase pT vector	delta-4 desaturase conversion efficiency	Gene dosage effect	Deduced MoA of 1st delta-4 desaturase	Deduced MoA of 2nd delta-4 desaturase
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	pT vector	28.8 ± 1.0	N/A	CoA	N/A
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	pT vector	27.0 ± 2.5	N/A	PC	N/A
c-d5Elo(Ot_GA3)	c-d4Des(Pl_GA)2	c-d4Des(Tc_GA)	44.0 ± 0.9	++ (0.79)	CoA	PC
c-d5Elo(Ot_GA3)	c-d4Des(Eg)	c-d4Des(Tc_GA)	29.4 ± 0.6	-	PC	PC
c-d5Elo(Ot_GA3)	c-d4Des(Tc_GA)	c-d4Des(Tc_GA)	32.2 ± 0.8	-	PC	PC

*Cultures were fed with ARA and production of DTA (delta-5 elongation) and DPAn-6 (delta-4 desaturation) were measured. pY: pYES2.1/V5-His-TOPO expression vector; pL: pESC-Leu expression vector; pT: PESC-Trp expression vector. All genes were placed under control of the Gal1 promoter. N/A: not applicable. Dosage effect: number in brackets = conversion efficiency of two-desaturase construct/ (conversion efficiency of single gene A construct + conversion efficiency of single gene B construct).

The desaturation levels achieved by the *P. lutheri* and c-d4Des(Tc_GA)s were similar. When two delta-4 desaturases were used, a significant gene dosage effects only observed with the *P. lutheri/Thraustochyrium* sp. pair, and the lowest with two copies of the *Thraustocytrium* sp desaturase. These trends were observed in the presence of elongated ARA (Table 170) and EPA (Table 169). However, with the n-6 elongation substrate (ARA), the desaturation conversion percentage was generally slightly lower compared to the omega-3 substrate (EPA).

Example 23: Spacer Regions Containing Transcription Factor Binding Motifs

As indicated in examples 3 and 4, between each expression cassette in the constructs used in the invention there is a spacer region of 100-200 base pairs. For the BiBAC T-DNAs, the short stretches of sequence in between the gene cassettes, which had been multi-cloning sites and Gateway site sequences in the co-transformation vectors, were subjected to randomization to maintain the GC content and to remove multiple repeats of identical sequence. The skilled worker would recognize that the presence of multiple repeats within a construct, in particular large multi-gene constructs, could result in internal deletions and rearrangements as the plasmid was cloned into *Escherichia coli* and passaged through the *Agrobacterium* strain/species used. After removal of repeats and adjustment of GC content, the sequences of the spacer regions were then examined for any possible binding sites for transcription factors and other DNA

interacting proteins. The spacer region sequences used are RTP10690_1 through RTP10690_12, including the AtAHAS promoter and terminator, RTP10690_5', LJB2197_5' and LJB2197_1 through LJB2197_4. The underscore numbers also represent the order in which that spacer region appears in the cassette reading from the right border towards the left border, in the sense orientation of the GOI in the proceeding cassette. For example, RTP10690_1 was the sequence after the terminator of the first cassette and before the promoter of the second cassette in the construct VC-RTP10690-1qcz_F. The terminator of the AHAS selection cassette and the 5' region of the first cassette were also examined.

>RTP10690_1
TTAATTCAGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGCGTCACGTGAC
GTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCTTAGCAGATATTT

>RTP10690_2
TTACTGATTGTCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGGCTCACGTG
ACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTAGCCTCACG
TGACCTTAGCTAACACTAGGTAGCGTCAGCTCGACGGCCCG

>RTP10690_3
GGCGGAGTGGGGCTACGTAGCGTCACGTGACGTTACCTAAGCCTAGGTAGCCTCAGC
TGACGTTACGTAACGCTAGGTAGGCTCAGCTGACACGGGCAGGACATAG

>RTP10690_4
GAATGAAACCGATCTACGTAGGCTCAGCTGAGCTTAGCTAAGCCTACCTAGCCTCACG
TGAGATTATGTAAGGCTAGGTAGCGTCACGTGACGTTACCTAACACTAGCTAGCGTCA
GCTGAGCTTAGCTAACCCTACGTAGCCTCACGTGAGCTTACCTAACGCTACGTAGCCT
CACGTGACTAAGGATGACCTACCCATTCTT

>RTP10690_5
GCGGCCGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGCGTCACGTGACG
TTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCCACGGGCAGGACATA
GGGACTACTACAA

>RTP10690_6

CGAATGAAACCGATCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGGCTCAC
GTGACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTAGCCTC
ACGTGACCTTAGCTAACACTAGGTAGCGTCAGCTTAGCAGATAT

>RTP10690_7
CCGTTCAATTTACTGATTGTCTACGTAGCGTCACCTGACGTTACGTAAGGCTACCTAGG
CTCACGTGACGTTACGTAACGCTACGTAGCGTCAGGTGAGGTTAGCTAACGCTAGCTA
GCCTCACCTGACGTTAGGTAAGGCTACGTAGCGTCACCTGAGATTAGCTAAGCCTACC
TAGACTCACGTGACCTTAGGTAACGCTACGTAGCGTCAAAGCTTTACAACGCTACACA
AA

>RTP10690_8
TTACCTAACTTAGAACTAAAATCAACTCTTTGTGACGCGTCTACCTAGAGTCAGCTGAG
CTTAGCTAACGCTAGCTAGTGTCAGCTGACGTTACGTAAGGCTAACTAGCGTCACGTG
ACCTTACGTAACGCTACGTAGGCTCAGCTGAGCTTAGCTAACCCTAGCTAGTGTCACG
TGAGCTTACGCTACTATAGAAAATGTGTTATAT

>RTP10690_9
TTCAGTCTAAAACAACTACGTAGCGTCACGTGACGTTACCTAAGCCTAGGTAGCCTCA
GCTGACGTTACGTAACGCTAGGTAGGCTCAGCTGACTGCAGCAAATTTACACATTGCC
A

>RTP10690_10
TAATTCGGCGTTAATTCAGCTACGTAGGCTCAGCTGAGCTTACCTAAGGCTACGTAGG
CTCACGTGACGTTACGTAAGGCTACGTAGCGTCACGTGAGCTTACCTAACTCTAGCTA
GCCTCACGTGACCTTAGCTAACACTAGGTAGCGTCAGCACAGATGAATACTAGCTGTT
GTTCA

>RTP10690_11
ATCATGATGCTTCTCTGAGCCGTGTTTGCTAGCTAGCCTCAGCTGACGTTACGTAACG
CTAGGTAGCGTCACGTGACGTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAA
GCGCACAGATGAATACTAGCTGTTGTTCACA

>RTP10690_12

CTTCTCTGAGCCGTGTTTGCTAGCTAGCCTCAGCTGACGTTACGTAACGCTAGGTAGC
GTCACGTGACGTTAGCTAACGCTAGGTAGCGTCAGCTGAGCTTACGTAAGCGCTTAAT
TAAAGTACTGATATCGGTACCAAATCGAATCCAAAAATTACGGATATGAATAT

>RTP10690_5'
ATTACAACGGTATATATCCTGCCAGTCAGCATCATCACACCAAAAGTTAGGCCCGAATA
GTTTGAAATTAGAAAGCTCGCAATTGAGGTCTACAGGCCAAATTCGCTCTTAGCCGTAC
AATATTACTCACCGGTGCGATGCCCCCCATCGTAGGTGAAGGTGGAAATTAATGGCGC
GCCTGATCACTGATTAGTAACTATTACGTAAGCCTACGTAGCGTCACGTGACGTTAGCT
AACGCTACGTAGCCTCAGCTGACGTTACGTAAGCCTACGTAGCGTCACGTGAGCTTAG
CTAACGCTACCTAGGCTCAGCTGACGTTACGTAACGCTAGCTAGCGTCACTCCTGCAG
CAAATTTACACA

>t-AtAHASL
GAGATGAAACCGGTGATTATCAGAACCTTTTATGGTCTTTGTATGCATATGGTAAAAAA
ACTTAGTTTGCAATTTCCTGTTTGTTTTGGTAATTTGAGTTTCTTTTAGTTGTTGATCTGC
CTGCTTTTTGGTTTACGTCAGACTACTACTGCTGTTGTTGTTTGGTTTCCTTTCTTTCAT
TTTATAAATAAATAATCCGGTTCGGTTTACTCCTTGTGACTGGCTCAGTTTGGTTATTGC
GAAATGCGAATGGTAAATTGAGTAATTGAAATTCGTTATTAGGGTTCTAAGCTGTTTTAA
CAGTCACTGGGTTAATATCTCTCGAATCTTGCATGGAAAATGCTCTTACCATTGGTTTTT
AATTGAAATGTGCTCATATGGGCCGTGGTTTCCAAATTAAATAAAACTACGATGTCATC
GAGAAGTAAAATCAACTGTGTCCACATTATCAGTTTTGTGTATACGATGAAATAGGGTA
ATTCAAAATCTAGCTTGATATGCCTTTTGGTTCATTTTAACCTTCTGTAAACATTTTTTCA
GATTTTGAACAAGTAAATCCAAAAAAAAAAAAAAAAATCTCAACTCAACACTAAATTATTT
TAATGTATAAAAGATGCTTAAAACATTTGGCTTAAAAGAAAGAAGCTAAAAACATAGAGA
ACTCTTGTAAATTGAAGTATGAAAATATACTGAATTGGGTATTATATGAATTTTTCTGATT
TAGGATTCACATGATCCAAAAAGGAAATCCAGAAGCACTAATCAGACATTGGAAGTAG
G
>LJB2197_5'
ACATACAAATGGACGAACGGATAAACCTTTTCACGCCCTTTTAAATATCCGATTATTCTA
ATAAACGCTCTTTTCTCTTAGGTTTACCCGCCAATATATCCTGTCAAACACTGATAGTTT
AAACTGAAGGCGGGAAACGACAATCTGATCACTGATTAGTAACTAAGGCCTTTAATTAA
TCTAGAGGCGCGCCGGGCCCCCTGCAGGGAGCTCGGCCGGCCAATTTAAATTGATAT
CGGTACATCGATTACGCCAAGCTATCAACTTTGTATAGAAAAGTTGCCATGATTACGCC
AAGCTTGGCCACTAAGGCCAATTTCGCGCCCTGCAGCAAATTTACACA

>LJB2197_1
CAATTTACTGATTGTGTCGACGCGATCGCGTGCAAACACTGTACGGACCGTGGCCTAA
TAGGCCGGTACCCAAGTTTGTACAAAAAAGCAGGCTCCATGATTACGCCAAGCTTGGC
CACTAAGGCCAATTTAAATCTACTAGGCCGGCCATCGACGGCCCGGACTGTA
>LJB2197_2
GGCGGAGTGGGGGGCGCCTACTACCGGTAATTCCCGGGATTAGCGGCCGCTAGTCT
GTGCGCACTTGTATCCTGCAGGTTAGGCCGGCCACACGGGCAGGACATAGGG
>LJB2197_3
AAACCGAATGAAACCGATGGCGCCTACCGGTATCGGTCCGATTGCGGCCGCTTAAAG
GGCGAATTCGTTTAAACACTGTACGGACCGTGGCCTAATAGGCCGGTACCACCCAGCT
TTCTTGTACAAAGTGGCCATGATTACGCCAAGCTTGGCCACTAAGGCCAATTTAAATCT
ACTAGGCCGGCCATAAGGATGACCTACCCATTCTT
>LJB2197_4
TTAATTCAGGGCCGGCCAAAGTAGGCGCCTACTACCGGTAATTCCCGGGATTAGCGG
CCGCTAGTCTGTGCGCACTTGTATCCTGCAGGTTAGGCCGGCCATTAGCAGATATTT

Table 171: Cassettes contained in the constructs VC-RTP10960-1qcz_F and VC-LJB2197-1qcz and the spacer regions in front of them, which was 5' of the promoter region. The bold lettering indicates the spacer, which corresponds to the Seq ID's listed above above.

VC-RTP10960-1qcz_F
p-VfUSP_684bp::i-Atss18_252bp::c-d6-Elo[Pp_GA2]::t-CaMV35S
RTP10690_1
p-LuCnl[1064bp]::i-Atss14_377bp::c-d5Des[Tc_GA2]::t-AgrOCS_192bp
RTP10690_2
p-SBP::i-Atss2_455bp::c-d6-Des[Ot_febit]::t-StCATHD-pA
RTP10690_3
p-LuPXR_1727bp::i-Atss1_847bp::c-d6-Elo[Tp_GA2]::t-AtPXR_400bp
RTP10690_4
p-Napin_A/B::i-Atss14_377bp::c-d12Des[Ps_GA2]::t-E9
RTP10690_5
p-LuPXR_1727bp::i-Atss15_758bp::c-

w3Des[pPir_GA]::AtPXR_400bp
RTP10690_6
p-LuCnl[1064bp]::i-Atss2_455bp::c-d4Des[Pl_GA]2::t-AgrOCS_192bp
RTP10690_7
p-BnFAE1::i-Atss1_847bp::c-d5Elo[Ot_GA3]::t-bnFAE1
RTP10690_8
p-ARC5_perm1::c-d4Des[Tc_GA3]::t-pvarc
RTP10690_9
p-VfUSP_684bp::i-Atss18_252::c-w3-Des[Pi_GA2]::t-CaMV35S
RTP10690_10
p-BnSETL-v1[1234bp]::c-d5-Des[Tc_GA2]::t-BnSETL
RTP10690_11
p-BnSETL-v1[1234bp]::c-w3Des[Pir_GA]::t-BnSETL
RTP10690_12
RTP10690_5'
t-AtAHASL
VC-LJB2197-1qcz
LJB2197_5'
p-VfUSP_684bp::i-Atss18_252bp::c-d6-Elo[PpGA2]::t-CaMV35S
LJB2197_1
p-LuCnl[1064bp]::i-Atss14_377bp::c-d5Des[Tc_GA2]::t-AgrOCS_192bp
LJB2197_2
p-SBP::i-Atss2_455bp::c-d6-Des[Ot_febit]::StCATHD-pA
LJB2197_3
p-LuPXR_1727bp::i-Atss1_847bp::c-d6-Elo[Tp_GA2]::t-AtPXR_400bp
LJB2197_4
p-Napin_A/B::i-Atss14_377bp::c-d12-Des[Ps_GA]::t-E9

Transcription factors are known to bind certain sequences of DNA and from this point of interaction with the chromosome they regulate transcription of a certain gene or subset of genes. The specific sequences which are bound by the transcription factor are referred to as DNA binding motifs and can be in the range of tens of base pairs to as few as four base pairs. The majority of DNA binding motifs consist of less than ten base pairs but those motifs have been documented to be necessary and sufficient for up (or down) regulation of transcription by the transcription factor binding to them. For examples of transcription factor motifs and the enabling data that are used to support the motifs see; Hattori et al., 2002, Keller et al., 1995, Kim et al., 2014, Lopez et al., 2013, Machens et al., 2014, Muino, 2013, Sarkar, 2013, Dubos et al., 2014). Identified motifs have been curated and archived in various data bases, such as PLACE (Higo et al., 1999), which can be accessed and queried by the public. While certain characterized promoters are used as the primary means to regulate expression of a given transgene in the invention, it was possible that alternative and unexpected regulation of a transgene was occurring in the plants described in the invention due to the presence of one or more active transcription factor binding motifs in the spacer region. Examples of artificial/synthetic promoters in the public literature support this line of reasoning; see Kong et al. (2014), Nishikata et al. (2013), and Brown et al. (2014) for examples of promoters made by random assembly of nucleotides and iterative testing as well as specific fragments of known transcriptional activity assembled to make an artificial promoter. Thus the regulatory region of a given transgene in the invention also includes, in addition to the intron, terminator and the promoter used, the spacer region in front of the cassette containing the transgene.

Table 172: lists the spacer region and the transcription factor binding motif present in the region and the nucleotide sequenceUpper case letters denote the motif while surrounding bases indicate examples of sequences that might neighbor the predicted motif but are not necessary for the motif to function in the regulation of transcription

Spacer ID	Detailed Family Information	Sequence
RTP10690_1	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_1	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_1	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_1	GT-box elements	gctgacGTTAcgtaacg
RTP10690_1	Cell-death specification 2	gttacGTAAcg
RTP10690_1	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc

RTP10690_1	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_1	Cell-death specification 2	gttacGTAAcg
RTP10690_1	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_1	GT-box elements	cctagcGTTAcgtaacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_1	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_1	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_1	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_1	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_1	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_1	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_1	ABA response elements	taacgtcaCGTGacgct
RTP10690_1	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_1	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_1	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_1	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_1	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_1	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_1	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_1	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_1	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgct
RTP10690_1	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_1	Cell-death specification 2	cttacGTAAgc
RTP10690_1	Plant G-box/C-box bZIP proteins	aagcgcttACGTaagctcagc
RTP10690_1	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_1	Cell-death specification 2	cttacGTAAgc
RTP10690_1	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgctta
RTP10690_1	Opaque-2 like transcriptional activators	gagcttACGTaagcgct
RTP10690_1	Vertebrate TATA binding protein factor	gcttacgTAAGcgctta
RTP10690_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	tatctgctaagcgctTACGtaagctca
RTP10690_1	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium	tctgctaagcgcttACGTaag
RTP10690_1	Arabidopsis CDC5 homolog	tgctaAGCGct
RTP10690_2	Cell-death specification 2	cttagGTAAgc
RTP10690_2	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_2	ABA response elements	taggctcaCGTGacgtt
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_2	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_2	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_2	Cell-death specification 2	gtaacGTCAcg
RTP10690_2	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_2	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_2	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg

RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_2	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_2	GT-box elements	cgtgacGTTAcgtaagg
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_2	Cell-death specification 2	cttacGTAAcg
RTP10690_2	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_2	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_2	Cell-death specification 2	gttacGTAAgg
RTP10690_2	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_2	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg
RTP10690_2	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_2	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_2	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_2	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_2	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_2	ABA response elements	taagctcaCGTGacgct
RTP10690_2	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_2	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_2	GT-box elements	cgtcacGTGAgcttacc
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_2	Cell-death specification 2	gttagGTAAgc
RTP10690_2	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_2	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_2	ABA response elements	tagcctcaCGTGacctt
RTP10690_2	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_2	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_2	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_2	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_2	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_2	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_2	GT-box elements	cctcacGTGAccttagc
RTP10690_2	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_2	Plant G-box/C-box bZIP proteins	tcgagcTGACgctacctagtg
RTP10690_2	Plant G-box/C-box bZIP proteins	actaggtaGCGTcagctcgac
RTP10690_3	Opaque-2 like transcriptional activators	gacgctACGTagcccca
RTP10690_3	Plant G-box/C-box bZIP proteins	gtggggctACGTagcgtcacg
RTP10690_3	Opaque-2 like transcriptional activators	ggggctACGTagcgtca
RTP10690_3	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtagcc
RTP10690_3	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgacg
Spacer ID	**Detailed Family Information**	**Sequence**

RTP10690_3	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_3	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacg
RTP10690_3	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_3	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_3	ABA response elements	taacgtcaCGTGacgct
RTP10690_3	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_3	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_3	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_3	Cell-death specification 2	gtaacGTCAcg
RTP10690_3	Plant G-box/C-box bZIP proteins	cttaggtaACGTcacgtgacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggcttaggtaacgtCACGtgacgcta
RTP10690_3	Opaque-2 like transcriptional activators	taggtaACGTcacgtga
RTP10690_3	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaagc
RTP10690_3	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_3	GT-box elements	cgtgacGTTAcctaagc
RTP10690_3	Cell-death specification 2	cttagGTAAcg
RTP10690_3	Vertebrate TATA binding protein factor	cgttaccTAAGcctagg
RTP10690_3	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_3	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_3	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_3	GT-box elements	gctgacGTTAcgtaacg
RTP10690_3	Cell-death specification 2	gttacGTAAcg
RTP10690_3	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_3	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_3	Cell-death specification 2	gttacGTAAcg
RTP10690_3	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_3	GT-box elements	cctagcGTTAcgtaacg
RTP10690_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacctagcgtTACGtaacgtca
RTP10690_3	Calmodulin binding / CGCG box binding proteins	gccCGTGtcagctgagc
RTP10690_3	Bel-1 similar region	tgtcctgcccgtgTCAGctgagc
RTP10690_4	Dehydration responsive element binding factors	gaatgaaaCCGAtctacgtag
RTP10690_4	Light responsive element motif, not modulated by different light qualities	cgATCTacgta
RTP10690_4	Plant G-box/C-box bZIP proteins	ataatctcACGTgaggctagg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taatctCACGtgaggctag
RTP10690_4	Opaque-2 like transcriptional activators	aatctcACGTgaggcta
RTP10690_4	ABA response elements	taatctcACGTgaggct
RTP10690_4	Plant G-box/C-box bZIP proteins	ctagcctcACGTgagattatg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgagattat
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgagatta
RTP10690_4	Brassinosteroid (BR) response element	cctcaCGTGagattatg
RTP10690_4	Cell-death specification 2	attatGTAAgg
RTP10690_4	Vertebrate TATA binding protein factor	gattatgTAAGgctagg
RTP10690_4	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcc
RTP10690_4	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg

RTP10690_4	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_4	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_4	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_4	ABA response elements	taacgtcaCGTGacgct
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_4	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_4	Cell-death specification 2	gtaacGTCAcg
RTP10690_4	Plant G-box/C-box bZIP proteins	gttaggtaACGTcacgtgacg
RTP10690_4	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttaggtaacgtCACGtgacgcta
RTP10690_4	Opaque-2 like transcriptional activators	taggtaACGTcacgtga
RTP10690_4	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaaca
RTP10690_4	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_4	GT-box elements	cgtgacGTTAcctaaca
RTP10690_4	Cell-death specification 2	gttagGTAAcg
RTP10690_4	Plant G-box/C-box bZIP proteins	ctcagcTGACgctagctagtg
RTP10690_4	Plant G-box/C-box bZIP proteins	actagctaGCGTcagctgagc
RTP10690_4	Telo box (plant interstitial telomere motifs)	gctaaCCCTacgtag
RTP10690_4	Opaque-2 like transcriptional activators	aaccctACGTagcctca
RTP10690_4	Brassinosteroid (BR) response element	gctcaCGTGaggctacg
RTP10690_4	Plant G-box/C-box bZIP proteins	gtaagctcACGTgaggctacg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	taagctCACGtgaggctac
RTP10690_4	Opaque-2 like transcriptional activators	aagctcACGTgaggcta
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcctcACGTgagcttacc
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgagcttac
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgagctta
RTP10690_4	Brassinosteroid (BR) response element	cctcaCGTGagcttacc
RTP10690_4	GT-box elements	cctcacGTGAgcttacc
RTP10690_4	Cell-death specification 2	gttagGTAAgc
RTP10690_4	MYB IIG-type binding sites	tagcgttaGGTAagc
RTP10690_4	Core promoter motif ten elements	cttacctAACGctacgtagcc
RTP10690_4	Plant G-box/C-box bZIP proteins	gtgaggctACGTagcgttagg
RTP10690_4	Opaque-2 like transcriptional activators	gaggctACGTagcgtta
RTP10690_4	Opaque-2 like transcriptional activators	aacgctACGTagcctca
RTP10690_4	Root hair-specific cis-elements in angiosperms	aacgctacgtagcctCACGtgacta
RTP10690_4	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aacgctacgtagcctCACGtgactaag
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_4	ABA response elements	tagcctcaCGTGactaa
RTP10690_4	Plant G-box/C-box bZIP proteins	ccttagtcACGTgaggctacg
RTP10690_4	Myc-like basic helix-loop-helix binding factors	cttagtCACGtgaggctac
RTP10690_4	Opaque-2 like transcriptional activators	ttagtcACGTgaggcta
RTP10690_4	Plant G-box/C-box bZIP proteins	gtagcctcACGTgactaagga
RTP10690_4	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgactaagg
RTP10690_4	Opaque-2 like transcriptional activators	agcctcACGTgactaag
RTP10690_4	GT-box elements	cctcacGTGActaagga
RTP10690_4	MYB IIG-type binding sites	aatgGGTAggtcatc
RTP10690_5	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg

RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaacg
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_5	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_5	GT-box elements	cctagcGTTAcgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_5	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_5	ABA response elements	taacgtcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_5	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_5	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgcc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_5	Cell-death specification 2	cttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	tggcgcttACGTaagctcagc
RTP10690_5	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_5	Cell-death specification 2	cttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgccac
RTP10690_5	Opaque-2 like transcriptional activators	gagcttACGTaagcgcc
RTP10690_5	Vertebrate TATA binding protein factor	gcttacgTAAGcgccac
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	cctgcccgtggcgctTACGtaagctca
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tgcccgtggcgctTACGtaag
RTP10690_5	Calmodulin binding / CGCG box binding proteins	gccCGTGgcgcttacgt
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcttacgtaagcgccACGGgcaggac

RTP10690_5	Plant G-box/C-box bZIP proteins	tgtcctgcCCGTggcgcttac
RTP10690_6	Dehydration responsive element binding factors	gaatgaaaCCGAtctacgtag
RTP10690_6	Light responsive element motif, not modulated by different light qualities	cgATCTacgta
RTP10690_6	Cell-death specification 2	cttagGTAAgc
RTP10690_6	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_6	ABA response elements	taggctcaCGTGacgtt
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_6	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_6	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_6	Cell-death specification 2	gtaacGTCAcg
RTP10690_6	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_6	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_6	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_6	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_6	GT-box elements	cgtgacGTTAcgtaagg
RTP10690_6	Cell-death specification 2	cttacGTAAcg
RTP10690_6	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_6	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_6	Cell-death specification 2	gttacGTAAgg
RTP10690_6	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_6	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg
RTP10690_6	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_6	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_6	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_6	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_6	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_6	ABA response elements	taagctcaCGTGacgct
RTP10690_6	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_6	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_6	GT-box elements	cgtcacGTGAgcttacc
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_6	Cell-death specification 2	gttagGTAAgc
RTP10690_6	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_6	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_6	ABA response elements	tagcctcaCGTGacctt

RTP10690_6	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_6	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_6	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_6	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_6	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_6	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_6	GT-box elements	cctcacGTGAccttagc
RTP10690_6	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_6	Plant G-box/C-box bZIP proteins	ctaagcTGACgctacctagtg
RTP10690_7	L1 box, motif for L1 layer-specific expression	aatCAGTaaattgaacg
RTP10690_7	Arabidopsis homeobox protein	caaTTTActga
RTP10690_7	Sucrose box	gaCAATcagtaaattgaac
RTP10690_7	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tttactgattgtctACGTagc
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aatttactgattgtcTACGtagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	gattgtctACGTagcgtcacc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Plant G-box/C-box bZIP proteins	gtcaggTGACgctacgtagac
RTP10690_7	Plant G-box/C-box bZIP proteins	tctacgtaGCGTcacctgacg
RTP10690_7	Plant G-box/C-box bZIP proteins	cgtagcgtcaccTGACgttac
RTP10690_7	Plant G-box/C-box bZIP proteins	cttacgtaACGTcaggtgacg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtcaccTGACgttacgtaagg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcacctgacgtTACGtaaggcta
RTP10690_7	Opaque-2 like transcriptional activators	cacctgACGTtacgtaa
RTP10690_7	GT-box elements	cctgacGTTAcgtaagg
RTP10690_7	Cell-death specification 2	cttacGTAAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcagg
RTP10690_7	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_7	Cell-death specification 2	gttacGTAAgg
RTP10690_7	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctaggtagcctTACGtaacgtca
RTP10690_7	ABA response elements	taggctcaCGTGacgtt
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctag
RTP10690_7	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_7	Plant G-box/C-box bZIP proteins	ctaggctcACGTgacgttacg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_7	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_7	Cell-death specification 2	gtaacGTCAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gttacgtaACGTcacgtgagc

RTP10690_7	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaacg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaacgcta
RTP10690_7	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_7	GT-box elements	cgtgacGTTAcgtaacg
RTP10690_7	Cell-death specification 2	gttacGTAAcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gtagcgttACGTaacgtcacg
RTP10690_7	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_7	Cell-death specification 2	gttacGTAAcg
RTP10690_7	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_7	GT-box elements	cgtagcGTTAcgtaacg
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcgtTACGtaacgtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Plant G-box/C-box bZIP proteins	ctgacgctACGTagcgttacg
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcgtta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgctACGTagcgtcagg
RTP10690_7	Opaque-2 like transcriptional activators	aacgctACGTagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaccTGACgctacgtagcg
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcaggtgagg
RTP10690_7	MYB-like proteins	caggtgagGTTAgctaa
RTP10690_7	Plant G-box/C-box bZIP proteins	cttacctaACGTcaggtgagg
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaccTGACgttaggtaagg
RTP10690_7	MYB IIG-type binding sites	tgacgttaGGTAagg
RTP10690_7	Cell-death specification 2	gttagGTAAgg
RTP10690_7	GT-box elements	cgttagGTAAggctacg
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacc
RTP10690_7	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_7	Plant G-box/C-box bZIP proteins	ctcaggTGACgctacgtagcc
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacctgaga
RTP10690_7	Myc-like basic helix-loop-helix binding factors	tagcgtCACCtgagattag
RTP10690_7	ABA response elements	tagactcaCGTGacctt
RTP10690_7	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgagtctagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgagtctag
RTP10690_7	Opaque-2 like transcriptional activators	aaggtcACGTgagtcta
RTP10690_7	TFF-box	ctAAGGtcacgtgagtctagg
RTP10690_7	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_7	Plant G-box/C-box bZIP proteins	ctagactcACGTgaccttagg
RTP10690_7	Myc-like basic helix-loop-helix binding factors	tagactCACGtgaccttag
RTP10690_7	Opaque-2 like transcriptional activators	agactcACGTgaccta
RTP10690_7	GT-box elements	actcacGTGAccttagg
RTP10690_7	Cell-death specification 2	cttagGTAAcg
RTP10690_7	GT-box elements	ccttagGTAAcgctacg
RTP10690_7	GT-box elements	cgtagcGTTAcctaagg
RTP10690_7	Plant G-box/C-box bZIP proteins	ttgacgctACGTagcgttacc
RTP10690_7	Opaque-2 like transcriptional activators	gacgctACGTagcgtta
RTP10690_7	Plant G-box/C-box bZIP proteins	gtaacgctACGTagcgtcaaa
RTP10690_7	Opaque-2 like transcriptional activators	aacgctACGTagcgtca
RTP10690_7	Plant specific NAC [NAM (no apical meristem), ATAF172,	gtaaagctttgacgctACGTagcgtta

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
RTP10690_7	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcaaagcttt
RTP10690_7	Calcium regulated NAC-factors	taaaGCTTtgacgctacgtag
RTP10690_7	W Box family	aagctTTGAcgctacgt
RTP10690_7	Tracheary-element-regulating cis-elements, conferring TE-specific expression	cgtcAAAGctt
RTP10690_7	Vertebrate TATA binding protein factor	cgttgTAAAgctttgac
RTP10690_7	DNA binding with one finger (DOF)	agcgttgtAAAGctttg
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_7	Mitochondral HMG-box transcription factor	tgtagcgTTGTaa
RTP10690_8	Secondary wall NACS	gattttagttcTAAGttag
RTP10690_8	AS1/AS2 repressor complex	gagTTGAtt
RTP10690_8	Calmodulin binding / CGCG box binding proteins	agaCGCGtcacaaagag
RTP10690_8	Coupling element 3 sequence	ggtagaCGCGtcacaaaga
RTP10690_8	Plant G-box/C-box bZIP proteins	aggtagacGCGTcacaaagag
RTP10690_8	Plant G-box/C-box bZIP proteins	tctttgTGACgcgtctaccta
RTP10690_8	Calmodulin binding / CGCG box binding proteins	tgaCGCGtctacctaga
RTP10690_8	Plant G-box/C-box bZIP proteins	gctagtgtcagcTGACgttac
RTP10690_8	Plant G-box/C-box bZIP proteins	cttacgtaACGTcagctgaca
RTP10690_8	Plant G-box/C-box bZIP proteins	gtcagcTGACgttacgtaagg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgtcagctgacgtTACGtaaggcta
RTP10690_8	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_8	GT-box elements	gctgacGTTAcgtaagg
RTP10690_8	Cell-death specification 2	cttacGTAAcg
RTP10690_8	Plant G-box/C-box bZIP proteins	ttagccttACGTaacgtcagc
RTP10690_8	Opaque-2 like transcriptional activators	agccttACGTaacgtca
RTP10690_8	Cell-death specification 2	gttacGTAAgg
RTP10690_8	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctagttagcctTACGtaacgtca
RTP10690_8	MYB-like proteins	tgacgctAGTTagcctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtcacgTGACgctagttagcc
RTP10690_8	ABA response elements	tagcgtcaCGTGacctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtaaggtcACGTgacgctagt
RTP10690_8	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgacgctag
RTP10690_8	Opaque-2 like transcriptional activators	aaggtcACGTgacgcta
RTP10690_8	ABA response elements	taaggtcaCGTGacgct
RTP10690_8	Dc3 promoter binding factors	gTCACgtgacc
RTP10690_8	Plant G-box/C-box bZIP proteins	ctagcgtcACGTgaccttacg
RTP10690_8	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgaccttac
RTP10690_8	Opaque-2 like transcriptional activators	agcgtcACGTgacctta
RTP10690_8	GT-box elements	cgtcacGTGAccttacg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttacgtaaggtCACGtgacgcta
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcacgtgacctTACGtaacgcta
RTP10690_8	Cell-death specification 2	gttacGTAAgg

RTP10690_8	Plant G-box/C-box bZIP proteins	gtagcgttACGTaaggtcacg
RTP10690_8	Opaque-2 like transcriptional activators	agcgttACGTaaggtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_8	Cell-death specification 2	cttacGTAAcg
RTP10690_8	Opaque-2 like transcriptional activators	gaccttACGTaacgcta
RTP10690_8	GT-box elements	cgtagcGTTAcgtaagg
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacgtagcgtTACGtaaggtca
RTP10690_8	Plant G-box/C-box bZIP proteins	ctgagcctACGTagcgttacg
RTP10690_8	Opaque-2 like transcriptional activators	gagcctACGTagcgtta
RTP10690_8	Telo box (plant interstitial telomere motifs)	gctaACCCtagctag
RTP10690_8	ABA response elements	tagtgtcACGTgagctt
RTP10690_8	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacactagc
RTP10690_8	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacactag
RTP10690_8	Opaque-2 like transcriptional activators	aagctcACGTgacacta
RTP10690_8	ABA response elements	taagctcaCGTGacact
RTP10690_8	Plant G-box/C-box bZIP proteins	ctagtgtcACGTgagcttacg
RTP10690_8	Myc-like basic helix-loop-helix binding factors	tagtgtCACGtgagcttac
RTP10690_8	Opaque-2 like transcriptional activators	agtgtcACGTgagctta
RTP10690_8	GT-box elements	tgtcacGTGAgcttacg
RTP10690_8	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tgtcacgtgagctTACGctac
RTP10690_8	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgtcacgtgagctTACGctactata
RTP10690_8	Cell-death specification 2	gtagcGTAAgc
RTP10690_8	Pollen-specific regulatory elements	ctataGAAAatgtgtta
RTP10690_9	CA-rich motif	agtctaaAACAactacgta
RTP10690_9	General transcription factor IID, GTF2D	tcagtctaaaacaactacgtagcgtcacg tGACGttacc
RTP10690_9	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtagtt
RTP10690_9	Plant G-box/C-box bZIP proteins	actacgtaGCGTcacgtgacg
RTP10690_9	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_9	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgacgctacg
RTP10690_9	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_9	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_9	ABA response elements	taacgtcaCGTGacgct
RTP10690_9	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttacc
RTP10690_9	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttac
RTP10690_9	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_9	Cell-death specification 2	gtaacGTCAcg
RTP10690_9	Plant G-box/C-box bZIP proteins	cttaggtaACGTcacgtgacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggcttaggtaacgtCACGtgacgcta
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_9	Opaque-2 like transcriptional activators	taggtaACGTcacgtga

RTP10690_9	Plant G-box/C-box bZIP proteins	gtcacgTGACgttacctaagc
RTP10690_9	Opaque-2 like transcriptional activators	cacgtgACGTtacctaa
RTP10690_9	GT-box elements	cgtgacGTTAcctaagc
RTP10690_9	Cell-death specification 2	cttagGTAAcg
RTP10690_9	Vertebrate TATA binding protein factor	cgttaccTAAGcctagg
RTP10690_9	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_9	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_9	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_9	GT-box elements	gctgacGTTAcgtaacg
RTP10690_9	Cell-death specification 2	gttacGTAAcg
RTP10690_9	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_9	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_9	Cell-death specification 2	gttacGTAAcg
RTP10690_9	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_9	GT-box elements	cctagcGTTAcgtaacg
RTP10690_9	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gagcctacctagcgtTACGtaacgtca
RTP10690_9	Upstream sequence element of U-snRNA genes	aaatttACACattgcca
RTP10690_10	Opaque-2 like transcriptional activators	gagcctACGTagctgaa
RTP10690_10	Cell-death specification 2	cttagGTAAgc
RTP10690_10	Opaque-2 like transcriptional activators	gagcctACGTagcctta
RTP10690_10	ABA response elements	taggctcaCGTGacgtt
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaacgtcACGTgagcctacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgagcctac
RTP10690_10	Opaque-2 like transcriptional activators	aacgtcACGTgagccta
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaggctcACGTgacgttacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taggctCACGtgacgttac
RTP10690_10	Opaque-2 like transcriptional activators	aggctcACGTgacgtta
RTP10690_10	Cell-death specification 2	gtaacGTCAcg
RTP10690_10	Plant G-box/C-box bZIP proteins	cttacgtaACGTcacgtgagc
RTP10690_10	Opaque-2 like transcriptional activators	tacgtaACGTcacgtga
RTP10690_10	Plant G-box/C-box bZIP proteins	ctcacgTGACgttacgtaagg
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcacgtgacgtTACGtaaggcta
RTP10690_10	Opaque-2 like transcriptional activators	cacgtgACGTtacgtaa
RTP10690_10	GT-box elements	cgtgacGTTAcgtaagg
RTP10690_10	Cell-death specification 2	cttacGTAAcg
RTP10690_10	Plant G-box/C-box bZIP proteins	gtagccttACGTaacgtcacg
RTP10690_10	Opaque-2 like transcriptional activators	agccttACGTaacgtca
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_10	Cell-death specification 2	gttacGTAAgg
RTP10690_10	Opaque-2 like transcriptional activators	gacgttACGTaaggcta
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtagcctTACGtaacgtca
RTP10690_10	Opaque-2 like transcriptional activators	gacgctACGTagcctta
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaaggctACGTagcgtcacg

RTP10690_10	Opaque-2 like transcriptional activators	aaggctACGTagcgtca
RTP10690_10	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtagcc
RTP10690_10	Plant G-box/C-box bZIP proteins	gctacgtaGCGTcacgtgagc
RTP10690_10	Plant G-box/C-box bZIP proteins	gtaagctcACGTgacgctacg
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_10	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_10	ABA response elements	taagctcaCGTGacgct
RTP10690_10	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttacc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttac
RTP10690_10	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_10	GT-box elements	cgtcacGTGAgcttacc
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agagttaggtaagctCACGtgacgcta
RTP10690_10	Cell-death specification 2	gttagGTAAgc
RTP10690_10	MYB IIG-type binding sites	tagagttaGGTAagc
RTP10690_10	MYB-like proteins	tagctagAGTTaggtaa
RTP10690_10	ABA response elements	tagcctcaCGTGacctt
RTP10690_10	Plant G-box/C-box bZIP proteins	ctaaggtcACGTgaggctagc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	taaggtCACGtgaggctag
RTP10690_10	Opaque-2 like transcriptional activators	aaggtcACGTgaggcta
RTP10690_10	Dc3 promoter binding factors	cTCACgtgacc
RTP10690_10	Plant G-box/C-box bZIP proteins	ctagcctcACGTgaccttagc
RTP10690_10	Myc-like basic helix-loop-helix binding factors	tagcctCACGtgaccttag
RTP10690_10	Opaque-2 like transcriptional activators	agcctcACGTgacctta
RTP10690_10	GT-box elements	cctcacGTGAccttagc
RTP10690_10	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agtgttagctaaggtCACGtgaggcta
RTP10690_10	Plant G-box/C-box bZIP proteins	ctgtgcTGACgctacctagtg
RTP10690_10	Plant G-box/C-box bZIP proteins	actaggtaGCGTcagcacaga
RTP10690_10	Myc-like basic helix-loop-helix binding factors	agtattCATCtgtgctgac
RTP10690_10	GAP-Box (light response elements)	acagATGAatactag
RTP10690_11	VIP1 responsive elements	acacgGCTCag
RTP10690_11	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_11	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_11	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_11	GT-box elements	gctgacGTTAcgtaacg
RTP10690_11	Cell-death specification 2	gttacGTAAcg
RTP10690_11	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_11	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_11	Cell-death specification 2	gttacGTAAcg
RTP10690_11	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_11	GT-box elements	cctagcGTTAcgtaacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_11	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_11	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg

RTP10690_11	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_11	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_11	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_11	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_11	ABA response elements	taacgtcaCGTGacgct
RTP10690_11	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_11	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_11	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_11	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_11	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_11	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_11	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_11	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_11	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgca
RTP10690_11	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
RTP10690_11	Cell-death specification 2	cttacGTAAgc
RTP10690_11	Plant G-box/C-box bZIP proteins	gtgcgcttACGTaagctcagc
RTP10690_11	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_11	Cell-death specification 2	cttacGTAAgc
RTP10690_11	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgcaca
RTP10690_11	Opaque-2 like transcriptional activators	gagcttACGTaagcgca
RTP10690_11	Vertebrate TATA binding protein factor	gcttacgTAAGcgcaca
RTP10690_11	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	attcatctgtgcgctTACGtaagctca
RTP10690_11	Plant G-box/C-box bZIP proteins	tcatctgtgcgcTTACgtaag
RTP10690_11	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	tcatctgtgcgctTACGtaag
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_11	Myc-like basic helix-loop-helix binding factors	agtattCATCtgtgcgctt
RTP10690_11	GAP-Box (light response elements)	acagATGAatactag
RTP10690_11	MYB-like proteins	tactagctGTTGttcac
RTP10690_12	VIP1 responsive elements	acacgGCTCag
RTP10690_12	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagg
RTP10690_12	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaacgcta
RTP10690_12	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_12	GT-box elements	gctgacGTTAcgtaacg
RTP10690_12	Cell-death specification 2	gttacGTAAcg
RTP10690_12	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_12	Opaque-2 like transcriptional activators	agcgttACGTaacgtca

RTP10690_12	Cell-death specification 2	gttacGTAAcg
RTP10690_12	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_12	GT-box elements	cctagcGTTAcgtaacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacctagcgtTACGtaacgtca
RTP10690_12	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacctagcg
RTP10690_12	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcacgtgacg
RTP10690_12	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_12	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacc
RTP10690_12	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_12	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_12	ABA response elements	taacgtcaCGTGacgct
RTP10690_12	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_12	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_12	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_12	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta
RTP10690_12	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_12	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_12	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_12	Plant G-box/C-box bZIP proteins	ctcagcTGACgctacctagcg
RTP10690_12	Plant G-box/C-box bZIP proteins	gctaggtaGCGTcagctgagc
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgtcagctgagctTACGtaagcgct
RTP10690_12	Vertebrate TATA binding protein factor	gcttacgTAAGctcagc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_12	Cell-death specification 2	cttacGTAAgc
RTP10690_12	Plant G-box/C-box bZIP proteins	aagcgcttACGTaagctcagc
RTP10690_12	Opaque-2 like transcriptional activators	gcgcttACGTaagctca
RTP10690_12	Cell-death specification 2	cttacGTAAgc
RTP10690_12	Plant G-box/C-box bZIP proteins	ctgagcttACGTaagcgctta
RTP10690_12	Opaque-2 like transcriptional activators	gagcttACGTaagcgct
RTP10690_12	Vertebrate TATA binding protein factor	gcttacgTAAGcgctta
RTP10690_12	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ctttaattaagcgctTACGtaagctca
RTP10690_12	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	ttaattaagcgcttACGTaag
RTP10690_12	Vertebrate TATA binding protein factor	ctttaatTAAGcgctta
RTP10690_12	DNA binding with one finger (DOF)	gcttaattAAAGtactg
RTP10690_12	Arabidopsis homeobox protein	gtaCTGAtatc
RTP10690_12	Dehydration responsive element binding factors	atttggtaCCGAtatcagtac
RTP10690_12	SBP-domain proteins	tatcgGTACcaaatcga
RTP10690_12	Sucrose box	aaAAATtacggatatgaat
RTP10690_12	MYB proteins with single DNA binding repeat	attcatATCCgtaattt
RTP10690_5	M-phase-specific activator elements	attacAACGgtatat

RTP10690_5	Yeast TATA binding protein factor	aacggtaTATAtcctgc
RTP10690_5	Yeast TATA binding protein factor	ggcaggaTATAtaccgt
RTP10690_5	Vertebrate TATA binding protein factor	cggtaTATAtcctgcca
RTP10690_5	MYB proteins with single DNA binding repeat	gtatatATCCtgccagt
RTP10690_5	Caenorhabditis maternal gene product SKN-1	cagcATCAtcaca
RTP10690_5	Fungal and oomycete pathogen response cluster - promoter motif	ctattcGGGCctaactt
RTP10690_5	Retroviral upstream element	ggcccgaatagTTTGaaatta
RTP10690_5	SBP-domain proteins	tagcCGTAcaatattac
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	gtgagtaatattgtACGGcta
RTP10690_5	Arabidopsis homeobox protein	aatATTActca
RTP10690_5	Opaque-2 like transcriptional activators	accggTGAGtaatattg
RTP10690_5	Epstein-Barr virus transcription factor R	atgccccccatcgtaGGTGaa
RTP10690_5	Retroviral PolyA signal	gCCATtaatttccaccttcac
RTP10690_5	Retroviral upstream element	cgcgccattaaTTTCcacctt
RTP10690_5	L1 box, motif for L1 layer-specific expression	cgcCATTaatttccacc
RTP10690_5	Arabidopsis homeobox protein	gaaATTAatgg
RTP10690_5	GT-box elements	gtaataGTTActaatca
RTP10690_5	MYB-like proteins	acgtaatAGTTactaat
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	cactgattagtaactatTACGtaagcc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ctgattagtaactatTACGtaagccta
RTP10690_5	Cell-death specification 2	cttacGTAAta
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaggcttACGTaatagttac
RTP10690_5	Cell-death specification 2	attacGTAAgc
RTP10690_5	Vertebrate TATA binding protein factor	tattacgTAAGcctacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtaggctTACGtaatagtt
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	actattacgtaagccTACGtagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaagcctACGTagcgtcacg
RTP10690_5	Opaque-2 like transcriptional activators	aagcctACGTagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgctacgtaggc
RTP10690_5	Plant G-box/C-box bZIP proteins	cctacgtaGCGTcacgtgacg
RTP10690_5	ABA response elements	tagcgtcaCGTGacgtt
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaacgtcACGTgacgctacg
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taacgtCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aacgtcACGTgacgcta
RTP10690_5	ABA response elements	taacgtcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgacgttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgacgttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgacgtta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttagctaACGTcacgtgacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaacgtCACGtgacgcta

RTP10690_5	Opaque-2 like transcriptional activators	tagctaACGTcacgtga
RTP10690_5	Plant G-box/C-box bZIP proteins	gtcacgTGACgttagctaacg
RTP10690_5	MYB-like proteins	cacgtgacGTTAgctaa
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgaggctACGTagcgttagc
RTP10690_5	Opaque-2 like transcriptional activators	gaggctACGTagcgtta
RTP10690_5	Opaque-2 like transcriptional activators	aacgctACGTagcctca
RTP10690_5	Plant G-box/C-box bZIP proteins	cttacgtaACGTcagctgagg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcctcagctgacgtTACGtaagccta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaagc
RTP10690_5	Cell-death specification 2	cttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaggcttACGTaacgtcagc
Spacer ID	**Detailed Family Information**	**Sequence**
RTP10690_5	Opaque-2 like transcriptional activators	aggcttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAgc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctgacgttACGTaagcctacg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaagccta
RTP10690_5	Vertebrate TATA binding protein factor	cgttacgTAAGcctacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gacgctacgtaggctTACGtaacgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	gtaagcctACGTagcgtcacg
RTP10690_5	Opaque-2 like transcriptional activators	aagcctACGTagcgtca
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcacgTGACgctacgtaggc
RTP10690_5	Plant G-box/C-box bZIP proteins	cctacgtaGCGTcacgtgagc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctaagctcACGTgacgctacg
RTP10690_5	Myc-like basic helix-loop-helix binding factors	taagctCACGtgacgctac
RTP10690_5	Opaque-2 like transcriptional activators	aagctcACGTgacgcta
RTP10690_5	ABA response elements	taagctcaCGTGacgct
RTP10690_5	Plant G-box/C-box bZIP proteins	gtagcgtcACGTgagcttagc
RTP10690_5	Myc-like basic helix-loop-helix binding factors	tagcgtCACGtgagcttag
RTP10690_5	Opaque-2 like transcriptional activators	agcgtcACGTgagctta
RTP10690_5	GT-box elements	cgtcacGTGAgcttagc
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcgttagctaagctCACGtgacgcta
RTP10690_5	Plant G-box/C-box bZIP proteins	gttacgtaACGTcagctgagc
RTP10690_5	Plant G-box/C-box bZIP proteins	ctcagcTGACgttacgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aggctcagctgacgtTACGtaacgcta
RTP10690_5	Opaque-2 like transcriptional activators	cagctgACGTtacgtaa
RTP10690_5	GT-box elements	gctgacGTTAcgtaacg
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Plant G-box/C-box bZIP proteins	ctagcgttACGTaacgtcagc
RTP10690_5	Opaque-2 like transcriptional activators	agcgttACGTaacgtca
RTP10690_5	Cell-death specification 2	gttacGTAAcg
RTP10690_5	Opaque-2 like transcriptional activators	gacgttACGTaacgcta
RTP10690_5	GT-box elements	gctagcGTTAcgtaacg
RTP10690_5	Plant specific NAC [NAM (no apical meristem), ATAF172,	gacgctagctagcgtTACGtaacgtca

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
RTP10690_5	Plant G-box/C-box bZIP proteins	caggagTGACgctagctagcg
RTP10690_5	Plant G-box/C-box bZIP proteins	gctagctaGCGTcactcctgc
RTP10690_5	Enhancer element first identified in the promoter of the octopine synthase gene (OCS) of the Agrobacterium tumefaciens T-DNA	ttgctgcaggagtGACGctag
t-AtAHASL	Retroviral PolyA signal	aCCATaaaaggttctgataat
t-AtAHASL	Vertebrate TATA binding protein factor	gaccaTAAAaggttctg
t-AtAHASL	Papillioma virus E2 transcriptional activator	agaccataaaAGGTtct
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	MADS box proteins	aaagaccatAAAAggttctga
t-AtAHASL	Papillioma virus E2 transcriptional activator	gaacctttaTGGTctt
t-AtAHASL	GT-box elements	ttttATGGtctttgtat
t-AtAHASL	MYB proteins with single DNA binding repeat	taccaTATGcatacaaa
t-AtAHASL	Arabidopsis homeobox protein	aaaCTTAgttt
t-AtAHASL	MYB-like proteins	aaaactTAGTttgcaat
t-AtAHASL	GT-box elements	gttttgGTAAtttgagt
t-AtAHASL	MYB-like proteins	ttcttttAGTTgttgat
t-AtAHASL	Retroviral CCAAT binding factors	aCCAAaaagcaggcagatcaacaac
t-AtAHASL	Sucrose box	aaAAAGcaggcagatcaac
t-AtAHASL	Legumin Box family	ttgatctGCCTgctttttggtttacgt
t-AtAHASL	DNA binding with one finger (DOF)	taaaccaaAAAGcaggc
t-AtAHASL	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	gcctgctttttggttTACGtcagacta
t-AtAHASL	Cell-death specification 2	ctgacGTAAac
t-AtAHASL	Plant G-box/C-box bZIP proteins	gtagtcTGACgtaaaccaaaa
t-AtAHASL	Cell-death specification 2	tttacGTCAga
t-AtAHASL	Plant G-box/C-box bZIP proteins	tttggtttACGTcagactact
t-AtAHASL	Plant G-box/C-box bZIP proteins	gcagtagtagtcTGACgtaaa
t-AtAHASL	Secondary wall NACS	tttataaaatgAAAGaaag
t-AtAHASL	Core promoter initiator elements	ttTCATtttat
t-AtAHASL	Vertebrate TATA binding protein factor	atttaTAAAatgaaaga
t-AtAHASL	Plant TATA binding protein factor	atttTATAaataaat
t-AtAHASL	Vertebrate TATA binding protein factor	ttttaTAAAtaaataat
t-AtAHASL	L1 box, motif for L1 layer-specific expression	tataaaTAAAtaatccg
t-AtAHASL	Retroviral PolyA signal	ataaaTAAAtaatccggttcg
t-AtAHASL	Dehydration responsive element binding factors	ggagtaaaCCGAaccggatta
t-AtAHASL	MYB-like proteins	tcagtttgGTTAttgcg
t-AtAHASL	GT-box elements	agtttgGTTAttgcgaa
t-AtAHASL	Cell-death specification 2	atttcGCAAta
t-AtAHASL	Cell-death specification 2	attgcGAAAtg
t-AtAHASL	Plant nitrate-responsive cis-elements	tcaatttaccattcgcatttcgcAATAac caaa
t-AtAHASL	TEF-box	gaATGGtaaattgagtaattg
t-AtAHASL	Cell-death specification 2	attgaGTAAtt
t-AtAHASL	MYB-like proteins	tgaaatTCGTtattagg
t-AtAHASL	Telo box (plant interstitial telomere motifs)	tagaaCCCTaataac
t-AtAHASL	Mitochondral HMG-box transcription factor	attagggTTCTaa
t-AtAHASL	VIP1 responsive elements	aaacaGCTTag
t-AtAHASL	CA-rich motif	ctgttaaAACAgcttagaa

t-AtAHASL	MYB-like proteins	cagtgaCTGTtaaaaca
t-AtAHASL	GT-box elements	cactggGTTAatatctc
t-AtAHASL	Circadian control factors	tgggttAATAtctct
t-AtAHASL	Myb-related DNA binding proteins (Golden2, ARR, Psr)	AGATtcgag
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	Legumin Box family	gcattttcCATGcaagattcgagagat
t-AtAHASL	GT-box elements	ttgcATGGaaaatgctc
t-AtAHASL	Retroviral upstream element	ggtaagagcatTTTCcatgca
t-AtAHASL	Mitochondral HMG-box transcription factor	aagagcaTTTTcc
t-AtAHASL	MYB proteins with single DNA binding repeat	tggaAAATgctcttacc
t-AtAHASL	LFY binding site	aACCAatggtaag
t-AtAHASL	CCAAT binding factors	aaCCAAtgg
t-AtAHASL	LFY binding site	tACCAttggtttt
t-AtAHASL	Retroviral PolyA signal	ttcaaTTAAaaaccaatggta
t-AtAHASL	Soybean embryo factor 4	ggTTTTtaatt
t-AtAHASL	DNA-binding proteins with the plant specific TCP-domain	cacgGCCCatatg
t-AtAHASL	Root hair-specific cis-elements in angiosperms	atttaatttggaaacCACGgcccat
t-AtAHASL	MADS box proteins	ggtTTCCaaattaaataaaac
t-AtAHASL	Vertebrate TATA binding protein factor	ttttattTAATttggaa
t-AtAHASL	Sulphur limitation, elements found in genes inducible during sulphur deprivation	agtttTATTtaatttggaaac
t-AtAHASL	High mobility group factors	gtttTATTtaatttg
t-AtAHASL	L1 box, motif for L1 layer-specific expression	ccaaatTAAAtaaaact
t-AtAHASL	Retroviral PolyA signal	ttaaaTAAAactacgatgtca
t-AtAHASL	Opaque-2 like transcriptional activators	actacgatgTCATcgag
t-AtAHASL	Caenorhabditis maternal gene product SKN-1	cgatGTCAtcgag
t-AtAHASL	Sucrose box	taAAATcaactgtgtccac
t-AtAHASL	Lentiviral Poly A downstream element	aaCTGTgtccacatt
t-AtAHASL	MYB-like proteins	acaaaactGATAatgtg
t-AtAHASL	Retroviral upstream element	cacattatcagTTTTgtgtat
t-AtAHASL	Core promoter initiator elements	taTCAGttttg
t-AtAHASL	GT-box elements	aataggGTAAttcaaaa
t-AtAHASL	Circadian control factors	taattcaaAATCtag
t-AtAHASL	Lentiviral Tata upstream element	aaggttaaaatgAACCaaaaggc
t-AtAHASL	GT-box elements	cagaaggTTAAaatgaa
t-AtAHASL	Heat shock factors	tttaacCTTCtgtaaac
t-AtAHASL	Nodulin consensus sequence 1	aAAATgtttac
t-AtAHASL	Circadian control factors	ttgttcaaAATCtga
t-AtAHASL	L1 box, motif for L1 layer-specific expression	aacaagTAAAtccaaaa
t-AtAHASL	Circadian control factors	aaaaaaaaAATCtca
t-AtAHASL	Mitochondral HMG-box transcription factor	ttgagatTTTTtt
t-AtAHASL	Arabidopsis homeobox protein	taaTTTAgtgt
t-AtAHASL	L1 box, motif for L1 layer-specific expression	caaCACTaaattatttt
t-AtAHASL	Storekeeper motif	cacTAAAttatttta
t-AtAHASL	Arabidopsis homeobox protein	aaaATAAttta
t-AtAHASL	Arabidopsis homeobox protein	taaATTAtttt
t-AtAHASL	Arabidopsis homeobox protein	tatttTAATgt
t-AtAHASL	Vertebrate TATA binding protein factor	atgtaTAAAagatgctt
t-AtAHASL	Nodulin consensus sequence 1	tAAAAgatgct
t-AtAHASL	Storekeeper motif	gctTAAAacatttgg
t-AtAHASL	Myc-like basic helix-loop-helix binding factors	ttaaaACATttggcttaaa
Spacer ID	**Detailed Family Information**	**Sequence**
t-AtAHASL	Sucrose box	ttAAAAcatttggcttaaa

t-AtAHASL	Secondary wall NACS	catttggcttaAAAGaaag
t-AtAHASL	Tracheary-element-regulating cis-elements, conferring TE-specific expression	cttaAAAGaaa
t-AtAHASL	Heat shock factors	taaaaacatagAGAAct
t-AtAHASL	DNA binding with one finger (DOF)	actcttgtAAATtgaag
t-AtAHASL	MYB proteins with single DNA binding repeat	cagtATATtttcatact
t-AtAHASL	MADS box proteins	taataCCCAattcagtatatt
t-AtAHASL	MADS box proteins	ataTACTgaattgggtattat
t-AtAHASL	CCAAT binding factors	acCCAAttc
t-AtAHASL	Sucrose box	ctAAATcagaaaaattcat
t-AtAHASL	MADS box proteins	tgaatcctaaatcaGAAAaat
UB2197_5	M-phase-specific activator elements	ggacgAACGgataaa
UB2197_5	MYB proteins with single DNA binding repeat	aggtttATCCgttcgtc
UB2197_5	Nodulin consensus sequence 1	gAAAAggttta
UB2197_5	DNA binding with one finger (DOF)	gggcgtgaAAAGgttta
UB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	acggataaacctttCACGcccttta
UB2197_5	E2F-homolog cell cycle regulators	ccttTTCAcgccctt
UB2197_5	Transposase-derived transcription factors	ttttCACGccctttaa
UB2197_5	Vertebrate TATA binding protein factor	atattTAAAagggcgtg
UB2197_5	DNA binding with one finger (DOF)	gatatttaAAAGggcgt
UB2197_5	Vertebrate TATA binding protein factor	cctttTAAAtatccgat
UB2197_5	Arabidopsis homeobox protein	ccgATTAttct
UB2197_5	Retroviral PolyA signal	tctaaTAAAcgctcttttctc
UB2197_5	Retroviral CCAAT binding factors	tCTAAtaaacgctcttttctcttag
UB2197_5	DNA binding with one finger (DOF)	ctaagagaAAAGagcgt
UB2197_5	Nodulin consensus sequence 2	tcttttCTCTtaggt
UB2197_5	Ethylen respone element factors	gGCGGgtaaacctaagaga
UB2197_5	DNA binding with one finger (DOF)	tggcgggtAAACctaag
UB2197_5	MYB proteins with single DNA binding repeat	caggATATattggcggg
UB2197_5	MYB proteins with single DNA binding repeat	caatatATCCtgtcaaa
UB2197_5	TALE (3-aa acid loop extension) class homeodomain proteins	tttGACAggatat
UB2197_5	W Box family	agtgtTTGAcaggatat
UB2197_5	E2F-homolog cell cycle regulators	tcgtTTCCcgccttc
UB2197_5	Circadian control factors	gaaacgacAATCtga
UB2197_5	MYB-like proteins	aggcctTAGTtactaat
UB2197_5	Vertebrate TATA binding protein factor	tagtaacTAAGgccttt
UB2197_5	Retroviral PolyA signal	attaaTTAAaggccttagtta
UB2197_5	DNA binding with one finger (DOF)	gattaattAAAGgcctt
UB2197_5	Arabidopsis homeobox protein	ttaatTAATct
UB2197_5	Arabidopsis homeobox protein	tagATTAatta
UB2197_5	Light responsive element motif, not modulated by different light qualities	taATCTagagg
Spacer ID	**Detailed Family Information**	**Sequence**
UB2197_5	Vertebrate TATA binding protein factor	caattTAAAttggccgg
UB2197_5	L1 box, motif for L1 layer-specific expression	tcaattTAAAttggccg
UB2197_5	Retroviral upstream element	accgatatcaaTTTAaattgg
UB2197_5	MYB proteins with single DNA binding repeat	accgaTATCaatttaaa
UB2197_5	Dehydration responsive element binding factors	tcgatgtaCCGAtatcaattt
UB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172,	tatcggtacatcgatTACGccaagcta

	CUC2 (cup-shaped cotyledons 2)] transcription factors	
LIB2197_5	Cell-death specification 2	ttggcGTAAtc
LIB2197_5	MADS box proteins	cttTTCTatacaaagttgata
LIB2197_5	Plant TATA binding protein factor	tttcTATAcaaagtt
LIB2197_5	Pollen-specific regulatory elements	gtataGAAAagttgcca
LIB2197_5	GT-box elements	aatcATGGcaacttttc
LIB2197_5	Arabidopsis homeobox protein	gtaATCAtggc
LIB2197_5	Arabidopsis homeobox protein	gccatgATTAc
LIB2197_5	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	aaaagttgccatgatTACGccaagctt
LIB2197_5	Cell-death specification 2	ttggcGTAAtc
LIB2197_5	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
LIB2197_5	E2F-homolog cell cycle regulators	caatTTCGcgccctg
LIB2197_1	Arabidopsis homeobox protein	caaTTTActga
LIB2197_1	Motifs of plastid response elements	cacgCGATcgcgtcgacacaatcagtaaatt
LIB2197_1	Calmodulin binding / CGCG box binding proteins	gatCGCGtcgacacaat
LIB2197_1	Core promoter motif ten elements	gcacgcgATCGcgtcgacaca
LIB2197_1	Calmodulin binding / CGCG box binding proteins	gatCGCGtgcaaacact
LIB2197_1	Root hair-specific cis-elements in angiosperms	tccgtacagtgtttgCACGcgatcg
LIB2197_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	ggtccgtacagtgtttgCACGcgatcg
LIB2197_1	SBP-domain proteins	ggtcCGTAcagtgtttg
LIB2197_1	SBP-domain proteins	ggccgGTACccaagttt
LIB2197_1	SBP-domain proteins	agtttGTACaaaaaagc
LIB2197_1	DNA binding with one finger (DOF)	tgtacaaaAAAGcaggc
LIB2197_1	Legumin Box family	caaaaaaGCAGgctccatgattacgcc
LIB2197_1	Arabidopsis homeobox protein	tccatgATTAc
LIB2197_1	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	agcaggctccatgatTACGccaagctt
LIB2197_1	Cell-death specification 2	ttggcGTAAtc
LIB2197_1	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
Spacer ID	**Detailed Family Information**	**Sequence**
LIB2197_1	L1 box, motif for L1 layer-specific expression	tagattTAAAttggcct
LIB2197_1	Circadian control factors	ccaatttaAATCtac
LIB2197_1	Vertebrate TATA binding protein factor	caattTAAAtctactag
LIB2197_1	Light responsive element motif, not modulated by different light qualities	aaATCTactag
LIB2197_2	E2F-homolog cell cycle regulators	gtaaTTCCcgggatt
LIB2197_2	Brassinosteroid (BR) response element	ctgccCGTGtggccggc
LIB2197_3	Core promoter initiator elements	ttTCATtcggt
LIB2197_3	Retroviral upstream element	ggcgccatcggTTTCattcgg
LIB2197_3	Papillioma virus E2 transcriptional activator	ggaccgatacCGGTagg
LIB2197_3	Papillioma virus E2 transcriptional activator	ctaccggtatCGGTccg
LIB2197_3	Dehydration responsive element binding factors	caatcggaCCGAtaccggtag

LIB2197_3	Core promoter motif ten elements	ggccgcaATCGgaccgatacc
LIB2197_3	TEF-box	taAAGGgcgaattcgtttaaa
LIB2197_3	SBP-domain proteins	ggtcCGTAcagtgttta
LIB2197_3	Activator-, mediator- and TBP-dependent core promoter element for RNA polymerase II transcription from TATA-less promoters	ggGTGGtaccg
LIB2197_3	SBP-domain proteins	ggccgGTACcacccagc
LIB2197_3	Soybean embryo factor 3	gtaccACCCagcttt
LIB2197_3	Arabidopsis homeobox protein	gtaATCAtggc
LIB2197_3	Arabidopsis homeobox protein	gccatgATTAc
LIB2197_3	Plant specific NAC [NAM (no apical meristem), ATAF172, CUC2 (cup-shaped cotyledons 2)] transcription factors	caaagtggccatgatTACGccaagctt
LIB2197_3	Cell-death specification 2	ttggcGTAAtc
LIB2197_3	Calcium regulated NAC-factors	ccaaGCTTggccactaaggcc
LIB2197_3	L1 box, motif for L1 layer-specific expression	tagattTAAAttggcct
LIB2197_3	Circadian control factors	ccaatttaAATCtac
LIB2197_3	Vertebrate TATA binding protein factor	caattTAAAtctactag
LIB2197_3	Light responsive element motif, not modulated by different light qualities	aaATCTactag
LIB2197_3	MYB IIG-type binding sites	aatgGGTAggtcatc
LIB2197_4	E2F-homolog cell cycle regulators	gtaaTTCCcgggatt

Of particular note in Table 172 was the surprisingly high number of sequences that contain abscisic acid related transcription factor binding motifs for the construct VC-RTP10690-1qcz_F. Abscisic acid was a plant hormone involved in seed maturation, desiccation, and stress response, see Cutler et al. (2010) for a comprehensive review. Based upon what was known about abscisic acid and its effect on seed maturation, the observed high number of motifs implicated in the regulation of gene expression in response to abscisic acid may have played a role in the observed transcript levels and timing of gene expression for the genes in VC-RTP10690-1qcz_F. In addition to the likely affects on transcription, it is also known in the literature that topography is affected by GC content (see Wachter et al., 2014) and it is prudent to have a GC content in the T-DNA matching that of the host as much as possible. In addition to reducing the possibility of deletions and rearrangements, reducing the amount of repeats would also impact DNA topography and gene regulation (Ramamoorthy et al. 2014). The spacer regions would contribute to the overall impact of the T-DNA upon the local chromatin structure, which in turn will impact how the genes in that region are regulated and transcribed (Parker et al. 2009, Meggendorfer et al. 2010)..

Example 24 Gene Expression, Copy number determination and event detection

Plant material representing wild type, the BiBAC VC-RTP10690-1qcz_F, and the co-transformation constructs: VC-LJB2197-1qcz with VC-LLM337-1qcz rc, VC-LJB2755-2qcz rc with VC-LLM391-2qcz rc and VC-LJB2755-2qcz rc with VC-LTM217-1qcz rc were harvested according to the date after pollination. Construct details (genes and promoters) are found inTable 1, Table 2, Table 4, Table 6, Table 7 and Table 8. Based on the day after pollination, which correlated with developmental stage under the growth conditions employed, the seeds were combined into pools. The pooled seeds were immediately frozen in liquid nitrogen and subsequently stored at -80° C. A third of the frozen immature seeds were homogenized using the Precelly®24-Dual technology in 7ml tubes and two ceramic beads (6500Hz, 2-3 times, 20sec). From each pool three aliquots of 50-70mg of tissue were used to isolate the RNA. Frozen tissue was ground to a fine powder and extracted following basic procedures familiar to one who was skilled in the art (see Ruuska et al. 2000 and Focks and Benning, 1998 as well as Sambrook et al.1989 for an overview on RNA extraction from immature seeds and sample and RNA handling). RNA was extracted according to the protocol "SG-MA_0007-2009 RNA isolation" using Spectrum Plant Total RNA-KIT part number STRN50 (SIGMA-ALDRICH GmbH, Munich, Germany). In average the concentration of total RNA was about 450ng/µl. The 260/280 ratio was at 2.2 and the 260/230 ratio at 2.3.

For cDNA synthesis for qPCR 1µg of total RNA was treated with DNAseI (DEOXYRIBUNUCLEASE I (AMP-D1, Amplification Grade from SIGMA-Aldrich, GmbH) according to the supplier's protocol. After DNAseI treatment, the reverse transcription reaction was performed with the SuperScript™ III First-Strand Synthesis SuperMix for qRT-PCR (Invitrogen, Cat. No. 11752-250) and with a combination of oligo dT and random hexamers to ensure thorough and even representation of all transcripts, regardless of length.

Quantitative real time pcr protocol

Transcript measurement by quantitative real time PCR was carried out using procedures considered standard to those skilled in the art; see Livak and Schmittgen (2001). The qPCR reactions were done as simplex TaqMan reactions. The endogenous

reference gene was isolated in house and used due to predicted stability of the transcript based on the observed stability of the transcript corresponding to the orthologue in *Arabidopsis thaliana* during development. The canola ortholog was isolated and the gene, SEQ ID, was part of the glycosyl-phosphatidyl inositol aminotransferase pathway (GPI). The cDNA reactions, described above, were diluted 1:4. 2µl cDNA, which corresponded to 25ng of total RNA, was used per 10µl qPCR reaction with JumpStart TAQ ReadyMix (P2893-400RXN Sigma-Aldrich, GmbH). Primer/probe concentrations were 900 nmol for forward and reverse primer and 100nmol TaqMan probe. The TaqMan probes for targets of interest were labeled with FAM/BHQ1, and the reference gene was labeled with Yakima Yellow/BHQ1.

Each qPCR assay included a 1:1 dilution curve (5 dilution steps) with cDNA from the pool VC-RTP10690-1qcz_F, a no template control, three -RT controls (VC-RTP10690-1qcz_F, VC-LTM593-1qcz rc (~4w) and co-transformation VC-LJB2197-1qcz + VC-LLM337-1qcz rc). From each pool three independent aliquots of cDNA were measured as technical repeats. The ABI PRISM® 7900 sequence detection system (Applied Biosystem) was used with the following PCR Conditions:

Initial denaturation	95°C for 300 seconds	1 cycle
Amplification	95°C for 15 seconds/60°C for 60 seconds repate	for 40 cycles

The raw data were the Ct values for the target and the endogenous reference gene, respectively. The dCt values were calculated by subtraction: Ct(GOI)-Ct(Ref). The Reference dCt value was set to equal zero, which was interpreted as meaning that if there was no difference between GPI and the gene of interest (dCt = 0) the expression was = 1. The fold expression was equal to 2^{-dCt} (where the dCt = (Ct(GOI)-Ct(Ref)-0)). Three samples from each pool were taken and the geomean as well as the geometric positive and negative deviation were calculated. The slopes of dilution curves were calculated for each gene of interest and the endogenous reference gene (GPI) as a measure for the amplification efficiency. Table 173,

Table 174 and Table 175 indicate the probes and primers used to amplify the genes for qPCR assays.

Table 173: Probes used in the qPCR reactions

Target of Interest	Probe	Probe Oligo
c-o3DES(Pi_GA2)	o3DES-594FAM	CGCTCACTTCTTCGTTGCTGGACTCTC
c-o3Des(Pir_GA)	o3DESPIR-198FAM	ATCATCTCTCTCGGAGTTC
c-o3Des(Pir_GA) 3'	o3DESPIR-962FAM	CGCTGCTCCTATCATCCCAACTTTCTTCA
j-o3DES(PIR_GA)/t-AtPXR	AtPXR-Fam	TCGACCTAGAGGATCCCCGGCC
j-BnSETLprom/o3DES(PIR_GA)	BnSETL-1186FAM	CTCTGCCAGCGACCAAATCGAAGC
j-o3DES(PIR_GA)/t-BnSETL	BnSETL-Fam	CCTTGTGTTAGTTTAATG
c-d5Elo(Ot_GA3)	E011	TGACAAACAAGCCACCAAGCCCAA
c-d4DES(TC_GA)	D4DES-Tc-FAM	TGCTTCCCCAATGTACGTTGCTAGGTTCT
c-d4Des(Eg_GA)	D4DES-Eg-FAM	AAGGCACATCCTCC
c-d4Des(PI_GA2)	D4DES-PI-770FAM	AGCTTCTTTTCTTGGACGCCCTTGAGC
GPI	Exp3-78-YAK	GGATTCGACATTCCATCGGCTTTGA

Table 174: Forward primers used in qPCR

Target of Interest	Forward Primer	Forward Primer Oligo
c-o3DES(Pi_GA2)	o3DES-572F	CCGCTGTGGTTATCTCTTTGC
c-o3Des(Pir_GA)	o3DESPIR-160F	CTTGGGAGGCTATGTATGTTAGAAGA
c-o3Des(Pir_GA) 3'	o3DESPIR-924F	GCTTTCCCTGAGCTTGTTAGGA
j-o3DES(PIR_GA)/t-AtPXR	o3DesPir-F	GGAGGCTAAGGCTGCTGCTA
j-	BnSETL-	GACCCCCTCTCTCTCGTTGTC

Target of Interest	Forward Primer	Forward Primer Oligo
BnSETLprom/o3DES(PIR_GA)	1164-F	
j-o3DES(PIR_GA)/t-BnSETL	BnSETL-1164-F	GACCCCCTCTCTCTCGTTGTC
c-d5Elo(Ot_GA3)	MA54	GCAATCGTTGGTAGCCATGA
c-d4DES(TC_GA)	D4DES-Tc-F	CAAATCGATGCTGAGTGCAGAT
c-d4Des(Eg_GA)	D4DES-EG-F	TGACAAGTAAGCCATCCGTCAGT
c-d4Des(Pl_GA2)	D4DES-Pl-746-F	CTGGTGAGGCTATGTACGCTTTT
GPI	Exp 3-52F	GATGAATATCCTCCTGATGCTAACC

Table 175: Reverse primers used for qPCR

Target of Interest	Reverse Primer	Reverse Primer Oligo
c-o3DES(Pi_GA2)	o3DES-652R	TCTTAAGTCCCAACTGGAGAGACA
c-o3Des(Pir_GA)	o3DESPIR-262R	AAACCAAGGAGCGTCAAGTCTAGA
c-o3Des(Pir_GA) 3'	o3DESPIR-1026-R	TCAACAACTCCGTACTTAGCGTACAT
j-o3DES(PIR_GA)/t-AtPXR	AtPXR-R	TGCATGTACGTTATATAGTAGGCTTTTG
j-BnSETLprom/o3DES(PIR_GA)	o3DESPIR-R	GCAGATGTAGAAGCCATGGTTTG
j-o3DES(PIR_GA)/t-BnSETL	o3DESPIR-R	GCAGATGTAGAAGCCATGGTTTG
c-d5Elo(Ot_GA3)	MA55	CGTGTACCACCACGCTTTGT
c-d4DES(TC_GA)	D4DES-Tc-988R	AACACGGTCAAAGCCTTCATAATC
c-d4Des(Eg_GA)	D4DES-Eg-R	ACTTTTCACCACCGACGAAGTT
c-d4Des(Pl_GA2)	D4DES-Pl-817R	CCTCCCACCTCCAAGCAA
GPI	Exp 3-	CTTGCATGATGATCAGGAAAGC

Target of Interest	Reverse Primer	Reverse Primer Oligo
	128R	

Transcript analysis of a time course for gene expression of the BiBAC and co-transformations.

Transformants from four constructs were assayed as described above and transcript abundance relative to a single standard was measured as described, following standard protocols known to those skilled in the art as well as instructions provided with the kits employed. The graphs in Figure 16, Figure 17, Figure 18, Figure 19, Figure 20 and Figure 21 represent abundance of the gene listed over time with the error represented by the geometric positive and negative deviation. The time was listed on the abscissa and represents days after pollination and the ordinate contains the expression of the gene of interest relative to an internal standard (canola GPI). Lines with two constructs listed represent co-transformation events.

The gene o3Des(Pi_GA2) driven by the VfUSP promoter with the CaMV35S terminator was present in VC-LJB2755-2qcz, VC-LLM337-1qcz rc and VC-RTP10960-1qcz_F. One observes in Figure 16 that VC-RTP10960-1qcz_F has relatively low transcript accumulation while the co-transformation combination of VC-LJB2755-2qcz and VC-LLM391-2qcz rc contains the highest accumulation. The overall trend for this transcript with the promoter, terminator, intron and spacer region in front of the promoter was to increase over seed development.

The gene d4Des(Pl_GA)2 driven by the LuCnl promoter with the AgrOCS 192bp[LED12] terminator was present in both VC-LTM217-1qcz rc and in VC-RTP10960-1qcz_F. One observes in Figure 19 for both constructs that the accumulation relative to the GPI reference was in the range of 4-12 fold with gradual accumulation over seed development, with VC-RTP10960-1qcz_F accumulating less than VC-LTM217-1qcz rc.

The o3Des(Pir_GA) gene was present in VC-LJB2755-2qcz and VC-RTP10960-1qcz_F driven by the LuCnl promoter with the AtPXR 400bp[LLL823] terminator while it was present in VC-LLM337-1qcz rc driven by the VfSBP_perm3 promoter with the StCATHD-pA terminator. Within error it was difficult to ascribe a pattern to any of the constructs, but as shown in Figure 17, single copy events of the construct combination VC-LJB2755-2qcz and VC-LLM391-2qcz had a peak towards seed maturation that was not observed with any of

the other constructs. An additional copy of the gene driven by the BnSETL promoter and terminated by the BnSETL terminator was present in RTP10960-1qcz_F. However, similar to the d5Des(Tc_GA2) gene controlled by the same set of control elements, the o3Des(Pir_GA) copy under control of the p-BnSETL promoter was expressed at lower levels than the other genes with different control elements (Figure 18).

The gene d4Des(Tc_GA) was contained in VC-LLM337-1qcz rc, RTP10960-1qcz_F and VC-LTM217-1qcz rc and was driven by the ARC5_perm1 promoter with the pvarc terminator. A single point mutant of this gene; d4Des(Tc_GA)_T564G was contained in VC-LLM391-2qcz rc and regulated by the ARC5_perm1 promoter with the pvarc terminator. The overall trend of expression with this spacer region, promoter and terminator combination was an increase over seed development and then a decrease towards seed maturation, as depicted in Figure 20. Interestingly, the construct pair VC-LJB2755-2qcz with VC-LLM391-2qcz rc did not adhere to this trend but rather continued to increase over seed development and had a more robust expression than the other constructs and construct combinations. As previously observed VC-RTP10960-1qcz_F had the lowest level of expression.

VC-LLM337-1qcz rc and VC-LLM391-2qcz rc contain d4Des(Eg_GA) driven by the LuCnI(1064) promoter with the AgrOCS 192bp[LED12] terminator. It can be seen in Figure 21, that similar expression levels that increased during seed development where observed of single copy events containing either one of these two constructs.

Copy Number

To assess whether the entire pathway for PUFA biosynthesis was being brought into the plant and to what extent duplication and/or deletion was occurring in selected events; copy analysis of certain genes along the T-DNA was carried out.

DNA extraction

Brown spotted immature seeds from three events were analyzed, in addition to cotyledons of germinating seedlings of two further events. The gDNA was isolated according to standard protocols consistent with Sambrook et al., 1982. To isolate plant tissue DNA the Wizard Magnetic 96 DNA Plant System was used (Promega, Madison, WI USA part number FF3760). The supplied protocols were followed with the listed changes being implemented in order to increase the amount of extracted DNA: the volume of the extraction buffer was

increased to two hundred microliters and the volume of beads used was increased to ninety microliters with the volume of elution buffer being decreased to twenty microliters.

Duplex qPCR reactions were performed using JumpStart TaqReadyMix for Quantitative PCR (Sigma, D7440). qPCR assays were validated according to standard protocols for qPCR, see Demeke and Jenkins (2010) and Livak and Schmittgen (2001). The genomic copy numbers were calculated with the ddCt method based on the mean of the reference dCt value of a pool of known single copy events. Using the Excel function "frequency", groups of 0, 1, 2, 3, >4 were counted both target and event wise. A Chi-square test was performed for testing the hypothesis the family was segregating for a single T-DNA. The cutoff of P value was set at > 0.0499 for a single T-DNA segregation.

PCR for copy number analysis was carried out using the primers and probes listed and according to standard protocols used by those skilled in the art, see Livak and Schmittgen (2001) and Demekes T. and Jenkins RG (2010).
The primer pairs and probes are listed by name below and sequences can be found in the sequence listing:

Target	Forward Primer	Reverse Primer	Probe
c-AHAS	c-AHAS_F	c-AHAS_R	c-AHAS_P
c-d4Des(Eg_GA)	c-d4Des(Eg_GA)_F	c-d4Des(Eg_GA)_R	c-d4Des(Eg_GA)_P
c-d4Des(Pl_GA)2	c-d4Des(Pl_GA)2_F	c-d4Des(Pl_GA)2_R	c-d4Des(Pl_GA)2_P
c-d4Des(Tc_GA)	c-d4Des(Tc_GA)_F	c-d4Des(Tc_GA)_R	c-d4Des(Tc_GA)_P
c-d5Des(Tc_GA)	c-d5Des(Tc_GA)_F	c-d5Des(Tc_GA)_R	c-d5Des(Tc_GA)_P
c-d5Elo(Ot_GA3)	c-d5Elo(Ot_GA3)_F	c-d5Elo(Ot_GA3)_R	c-d5Elo(Ot_GA3)_P
c-d6Des(Ot_febit)	c-d6Des(Ot_febit)_F	c-d6Des(Ot_febit)_R	c-d6Des(Ot_febit)_P
c-d6Elo(Pp_GA)	c-d6Elo(Pp_GA)_F	c-d6Elo(Pp_GA)_R	c-d6Elo(Pp_GA)_P
c-d6Elo(Tp_GA)	c-d6Elo(Tp_GA)_F	c-d6Elo(Tp_GA)_R	c-d6Elo(Tp_GA)_P
c-o3Des(Pi_GA)	c-o3Des(Pi_GA)_F	c-o3Des(Pi_GA)_R	c-o3Des(Pi_GA)_P
c-o3Des(Pir_GA)	c-o3Des(Pir_GA)_F	c-o3Des(Pir_GA)_R	c-o3Des(Pir_GA)_P
j-i-Atss1_c-d5Elo(Ot_GA3)	j-i-Atss1_c-d5Elo(Ot_GA3)_F	j-i-Atss1_c-d5Elo(Ot_GA3)_R	j-i-Atss1_c-d5Elo(Ot_GA3)_P
j-i-Atss1_c-d6Elo(Tp_GA2)	j-i-Atss1_c-d6Elo(Tp_GA2)_F	j-i-Atss1_c-d6Elo(Tp_GA2)_R	j-i-Atss1_c-d6Elo(Tp_GA2)_P
j-i-Atss14_c-d12Des(Ps_GA)	j-i-Atss14_c-d12Des(Ps_GA)_F	j-i-Atss14_c-d12Des(Ps_GA)_R	j-i-Atss14_c-d12Des(Ps_GA)_P
j-i-Atss18_c-d6Elo(Pp_GA2)	j-i-Atss18_c-d6Elo(Pp_GA2)_F	j-i-Atss18_c-d6Elo(Pp_GA2)_R	j-i-Atss18_c-d6Elo(Pp_GA2)_P
j-i-Atss18_c-o3Des(Pi_GA2)	j-i-Atss18_c-o3Des(Pi_GA2)_F	j-i-Atss18_c-o3Des(Pi_GA2)_R	j-i-Atss18_c-o3Des(Pi_GA2)_P
j-i-Atss2_c-d4Des(Tc_GA3)	j-i-Atss2_c-d4Des(Tc_GA3)_F	j-i-Atss2_c-d4Des(Tc_GA3)_R	j-i-Atss2_c-d4Des(Tc_GA3)_P
j-i-Atss2_c-d6Des(Ot febit_GA)	j-i-Atss2_c-d6Des(Ot febit_GA)_F	j-i-Atss2_c-d6Des(Ot febit_GA)_R	j-i-Atss2_c-d6Des(Ot febit_GA)_P
j-p-BnFAE_t-PvARC	j-p-BnFAE_t-PvARC_F	j-p-BnFAE_t-PvARC_R	j-p-BnFAE_t-PvARC_P

Target	Forward Primer	Reverse Primer	Probe
j-p-BnSETL-v1_c-o3Des(Pir_GA)	j-p-BnSETL-v1_c-o3Des(Pir_GA)_F	j-p-BnSETL-v1_c-o3Des(Pir_GA)_R	j-p-BnSETL-v1_c-o3Des(Pir_GA)_P
j-p-LuPXR_i-Atss15	j-p-LuPXR_i-Atss15_F	j-p-LuPXR_i-Atss15_R	j-p-LuPXR_i-Atss15_P
j-p-PvARC5_t-BnFAE	j-p-PvARC5_t-BnFAE_F	j-p-PvARC5_t-BnFAE_R	j-p-PvARC5_t-BnFAE_P
j-p-PvARC5_t-BnSETL	j-p-PvARC5_t-BnSETL_F	j-p-PvARC5_t-BnSETL_R	j-p-PvARC5_t-BnSETL_P
j-t-CaMV_p-LuCnl-2	j-t-CaMV_p-LuCnl-2_F	j-t-CaMV_p-LuCnl-2_R	j-t-CaMV_p-LuCnl-2_P
j-t-E9-p3-2	j-t-E9-p3-2_F	j-t-E9-p3-2_R	j-t-E9-p3-2_P
j-t-PvARC-p-LuCnl	j-t-PvARC-p-LuCnl_F	j-t-PvARC-p-LuCnl_R	j-t-PvARC-p-LuCnl_P
j-t-StCAT_p2_p-LuPXR	j-t-StCAT_p2_p-LuPXR_F	j-t-StCAT_p2_p-LuPXR_R	j-t-StCAT_p2_p-LuPXR_P
reference (Adh1)	reference (Adh1)_F	reference (Adh1)_R	reference (Adh1)_P
c-o3Des(Pi_GA2_SNP)	c-o3Des(Pi_GA2_SNP)_F	c-o3Des(Pi_GA2_SNP)_R	c-o3Des(Pi_GA2_SNP)_P
j-t-PvARC-p3	j-t-PvARC-p3_F	j-t-PvARC-p3_R	j-t-PvARC-p3_P
j-i-Atss15_c-o3Des(Pi_GA2)	j-i-Atss15_c-o3Des(Pi_GA2)_F	j-i-Atss15_c-o3Des(Pi_GA2)_R	j-i-Atss15_c-o3Des(Pi_GA2)_P
j-p-VfSBPperm3_c-o3Des(Pir_GA)	j-p-VfSBPperm3_c-o3Des(Pir_GA)_F	j-p-VfSBPperm3_c-o3Des(Pir_GA)_R	j-p-VfSBPperm3_c-o3Des(Pir_GA)_P
c-d15Des(Ch_ERTp_GA)	c-d15Des(Ch_ERTp_GA)_F	c-d15Des(Ch_ERTp_GA)_R	c-d15Des(Ch_ERTp_GA)_P
j-i-Atss2_c-d4Des(Pl_GA)-195R)	j-i-Atss2_c-d4Des(Pl_GA)-195R)_F	j-i-Atss2_c-d4Des(Pl_GA)-195R)_R	j-i-Atss2_c-d4Des(Pl_GA)-195R)_P

For Event specific detection the following protocol was used:

Preparation of the Jumpstart™ Taq ReadyMix (Sigma, P2893) for use with the Quantstudio™ 12K Flex Real-Time PCR system from Life Technologies

Prior to use, add to the Jumpstart™Taq ReadyMix :

$MgCl_2$ to a final concentration of 11mM and

ROX (=Sulforhodamine 101) to a final concentration of 120 nM

= prepared jumpstart mix (1)

Preparation of the final reaction mixture for duplex qPCR reactions.

The qPCR reactions are done in a 384-well plate (Life technologies, catalogue number 4309849) in a total volume of 10 microliters per well. Per reaction, 2µl of template DNA is mixed with 8 microliters of qPCR reaction mixture according to the table below. The plates are sealed with MicroAmp® Optical Adhesive Film (Life Technologies, catalogue number 4311971).

reaction components	Final concentration
Prepared jumpstart mix (1)	1x
Transgene target forward primer	900 nM
Transgene target reverse primer	900 nM
Transgene target probe	100 nM
Endogenous reference gene forward primer	900 nM

Endogenous reference gene reverse primer	900 nM
Endogenous reference gene probe	100 nM
Template DNA	2 µl
Nuclease free water	Add up to 10µl

The primers and probes for event specific detection are shows in Table 176.

Table 176: Primers and Probes used for event specific detection.

Event / Locus	Forward Primer	Reverse Primer	Probe
LANBCH locus 1 (N5)	LANBCH locus 1 (N5)_Forward primer	LANBCH locus 1 (N5)_Reverse primer	LANBCH locus 1 (N5)_Probe
LANBCH locus 1 (N9)	LANBCH locus 1 (N9)_Forward primer	LANBCH locus 1 (N9)_Reverse primer	LANBCH locus 1 (N9)_Probe
LANBCH locus 2 (N5)	LANBCH locus 2 (N5)_Forward primer	LANBCH locus 2 (N5)_Reverse primer	LANBCH locus 2 (N5)_Probe
LANBCH locus 2 (N9)	LANBCH locus 2 (N9)_Forward primer	LANBCH locus 2 (N9)_Reverse primer	LANBCH locus 2 (N9)_Probe
LBFDAU locus 1	LBFDAU locus 1_Forward primer	LBFDAU locus 1_Reverse primer	LBFDAU locus 1_Probe
LBFDAU locus 2	LBFDAU locus 2_Forward primer	LBFDAU locus 2_Reverse primer	LBFDAU locus 2_Probe
LBFDGG	LBFDGG_Forward primer	LBFDGG_Reverse primer	LBFDGG_Probe
LBFDHG locus 1	LBFDHG locus 1_Forward primer	LBFDHG locus 1_Reverse primer	LBFDHG locus 1_Probe
LBFDHG locus 2	LBFDHG locus 2_Forward primer	LBFDHG locus 2_Reverse primer	LBFDHG locus 2_Probe
LBFGKN	LBFGKN_Forward primer	LBFGKN_Reverse primer	LBFGKN_Probe
LBFIHE	LBFIHE_Forward primer	LBFIHE_Reverse primer	LBFIHE_Probe
LBFLFK locus 1	LBFLFK locus 1_Forward primer	LBFLFK locus 1_Reverse primer	LBFLFK locus 1_Probe
LBFLFK locus 2	LBFLFK locus 2_Forward primer	LBFLFK locus 2_Reverse primer	LBFLFK locus 2_Probe
LBFPRA Locus 1	LBFPRA Locus 1_Forward primer	LBFPRA Locus 1_Reverse primer	LBFPRA Locus 1_Probe
LBFPRA locus 2	LBFPRA locus 2_Forward primer	LBFPRA locus 2_Reverse primer	LBFPRA locus 2_Probe
LBFPRA Locus 3e	LBFPRA Locus 3e_Forward primer	LBFPRA Locus 3e_Reverse primer	LBFPRA Locus 3e_Probe

PCR conditions for event specific detection were as follows:

The qPCR is performed on the QuantStudio™ 12K Flex Real-Time PCR System (Life technologies) with following qPCR conditions:

step1: 95°C 5min

step2: 45 cycles

95°C, 18 sec

60°C, 1 min

Expected amplicon sizes are given below in base pairs (plus or minus 10 bp):

Amplicon	Amplicon Size plus or minus 10 bp

Amplicon LANBCH locus 1 (N5)	100 bp
Amplicon LANBCH locus 1 (N9)	150 bp
Amplicon LANBCH locus 2 (N5)	150 bp
Amplicon LANBCH locus 2 (N9)	120 bp
Amplicon LBFDAU locus 1	110 bp
Amplicon LBFDAU locus 2	95 bp
Amplicon LBFDGG	100 bp
Amplicon LBFDHG locus 1	200 bp
Amplicon LBFDHG locus 2	140 bp
Amplicon LBFGKN	140 bp
Amplicon LBFIHE	110 bp
Amplicon LBFLFK locus 1	160 bp
Amplicon LBFLFK locus 2	170 bp
Amplicon LBFPRA Locus 1	570 bp
Amplicon LBFPRA locus 2	190 bp
Amplicon LBFPRA Locus 3e	90 bp

Example 25 Protein levels

Pooled seed material from four week old siliques was used for protein extraction. To enrich the membrane proteins and reduce the background signal, microsomes isolated from ground seed tissue were used to analyze protein levels for the following enzymes: c-o3Des(Pi_GA2), c-o3Des(Pir_GA), d4Des(Eg), c-d4Des(Pl_GA)2, and c-d6Elo(Tp_GA2). For c-o3Des(Pir_GA) and c-d6Elo(Tp_GA2), the microsomes were further extracted with detergent and used for ELISA. The various versions of a given gene (denoted as GA, GA2 etc.) all encoded an identical protein corresponding to the gene listed.

Initially, in a mortar cooled with liquid nitrogen, seeds were ground to a fine powder in Extraction buffer (25 mM Tris, pH 7.4; 140 mM NaCl; 3 mM KCl; 3 mM DTT; 200 mM Sorbitol; and EDTA-free Protease inhibitors (Roche)) at a ratio of 1g seed/4 ml buffer. The plant cell-debris was removed by centrifugation (5000 x g, 10 minutes, 4 °C), re-extracted by grinding a second time as described above with 1.5 ml of Extraction Buffer, and then again separated by centrifugation (5000 x g, 10 minutes, 4 °C). The supernatant from these two extractions was combined and represents the cell-free extract (typically 3.5 ml/g seed). Microsomal pellets were obtained from the cell-free extracts by ultra-centrifugation (100,000 x g, 60 minutes, 4 °C). For the c-o3Des(Pi_GA2), d4Des(Eg), c-d4Des(Pl_GA)2, enzymes the microsomal pellets were suspended in Suspension Buffer (1xTBST+1%TritonX-100). 1X

TBST consists of 50 mM Tris, 150 mM NaCl, 0.05% tween 20 detergent with a final pH of 7.6 at 4 °C.

For c-o3Des(Pir_GA) and c-d6Elo(Tp_GA2) the microsomal fractions were isolated as described above, and proteins were solubilized with 1X TBST buffer and detergent of choice (For details, please see the table below). For c-d4Des(Tc_GA), c-d5Elo(Ot_GA3), c-d5Des(Tc_GA2), c-d6Des(Ot_febit), c-d6Elo(Pp_GA2), c-d12Des(Ps_GA), the seed powder was further ground in liquid nitrogen and extracted with 1xTBST+1%Triton X100+Protease Inhibitor at 1:3 W/V ratio. The supernatant was cleared at 13,000g for 20 minutes at 4°C using a micro-centrifuge and followed by a 10 minute spin at 13,000g. The clarified supernatant was used for ELISA and total protein assay.

Antibody/antigen hybridization conditions: Plates were blocked with 300 µl/well of freshly made blocking solution. The plates were sealed and incubated for 1 hour with continuous shaking. After 1 hour of incubation, the plates were washed twice with 1X TBST.
The extract from above was used in the ELISA assay. The plate was incubated with 100 µl of standards or sample extract at 4 °C for overnight, without shaking. Standards were serial diluted 1:3. The plates were sealed and incubated overnight at 4 °C. After the plates were incubated overnight, the plates were washed five times with 1X TBST. After the wash the plates were incubated with 100 µl of detecting antibody in blocking solution for 1 hour, sealed with continuous shaking. The plates were washed five times with 1X TBST at the end of the one hour incubation step. The plates were then incubated with 100 ml of HRP-conjugated donkey anti-animal of the detecting antibody secondary antibody (1:10,000) in blocking solution for 1 hour, in a sealed plate with continuous shaking. The plates were then washed five times with 1X TBST at the end of the blocking step. 100 µl of 1-step TMB was then added and the plates incubated at room temperature for 20 minutes, sealed with continuous shaking. After the twenty minutes, 100 µl of a 1M HCl solution was added and the plates read at 450 nm.

For c-d6Elo(Tp_GA2) only: all steps were the same as for the other proteins but in place of the HRP conjugated donkey anti-body system used for the others, 100 µl of biotin-conjugated donkey anti-rabbit IgG diluted 1:40,000 in 2% BSA/1X TBST was used. The plate was sealed and shaken continuously during this incubation step, which was done for one hour. The plate was washed five times with 1X TBST at the end of this step. To read this plate, the plate was incubated with 100 µl extravidin-peroxidase diluted 1:40,000 in 2%

BSA/1X TBST, sealed and incubated for 1 hour with continuous shaking. The plate was then washed five times with 1X TBST. The plate was read at 450 nm.

Table 177: Overview of hybridization for protein detection and quantification TMB (3,3',5,5'-tetramethylbenzidine) was the substrate for the horse radish peroxidase employed to visualize protein bands, supplied by Sigma-Aldrich T-0565 and used according to the instructions of the manufacturer.

	Coating AB	Blocking Buffer	Sample extraction buffer	Standard ID and dilution	Detecting AB	Secondary AB	TMB
o3Des(Pir)	AB212, 1:5,500	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 90 ng/ml, full length o3Des(Pir)	AB210 1:1,300, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d4Des(Tc)	AB216, 1:15,000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, RN121	AB217, 1:15,000, in blocking buffer	1:10,000 HRP-donkey anti Rabbit	1 step TMB, 20 min
d5Elo(ot)	AB214, 1:1500,	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, RN134	AB215, 1:1500, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d5Des (Tc)	AB90, 1:8000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 100 ng/ml, full lenth d5Des	AB 100, 1:40000, in blocking buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d6Des(Ot)	AB113, 1:500	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 100 ng/ml, full length d6Des(Ot)	AB 112 rabbit, 1:10000, in blocking buffer	1:10,000 HRP-donkey anti rabbit	1 step TMB, 20 min
d6Elo(Pp)	AB 107, 1:1000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 110 ng/ml, PA534	AB 121 chicken, 1:5000 in extraction buffer	1:10,000 HRP-donkey anti chicken	1 step TMB, 20 min
d6Elo(Tp)	AB125, 1:2000,	2% BSA in TBST	1 XTBST+1% DDM+Protease inihibitor	1:3, from 200 ng/ml, PA678	AB140, 1:1000, in blocking buffer	1:40,000 biotin-donkey anti rabbit for 1hr, then 1:40,000 extravidin-peroxidase	1 step TMB, 20 min
d12Des (Ps)	AB128, 1:3000	1% BSA in TBST	1% Triton X-100, + 1 xTBS, + Protease Inhibitor	1:3, from 200 ng/ml, PA557	AB 129, 1:3000, in blocking buffer	1:10,000 HRP-donkey anti Rabbit	1 step TMB, 20 min

Table 178: Levels of the proteins of interest were expressed as nanograms (ng) per milligram of total protein. The values were the average and standard deviation from three technical measurements of approximately four week old seed material. As one observes the construct VC-RTP10690-1qcz_F had overall less protein for the expressed genes than the combination of VC-LJB2197-1qcz and VC-LLM337-1qcz rc.

Protein	Wt	LJB2197+LLM337	VC-RTP10690-1qcz_F
c-o3Des(Pir_GA), ng/mg total microsomal protein	9.04 ± 0.5	387.5 ± 10.5	69.7 ± 1.2
c-d4Des(Tc_GA), ng/mg of total protein	0.04 ± 0.01	0.74 ± 0.03	0.1 ± 0.01
c-d5Elo(Ot_GA3), ng/mg of total protein	0.12 ± 0.01	2.85 ± 0.02	1.23 ± 0.01
c-d5Des(Tc_GA2), ng/mg of total protein	0.05 ± 0.01	2.03 ± 0.08	1.54 ± 0.03
c-d6Des(Ot_febit),ng/mg total protein	0 ± 0	4.1 ± 0.1	1.83 ± 0.01
c-d6Elo(Pp_GA2), ng/mg total protein	0 ± 0	3.77 ± 0.3	1.1 ± 0.01
c-d6Elo(Tp_GA2), ng/mg of total microsomal protein	0 ± 0	0.33 ± 0.06	0.38 ± 0.04
c-d12Des(Ps_GA), ng/mg of total protein	0.05 ± 01	1.5 ± 0.07	0.34 ± 0.03

Example 26: Desaturase and Elongase substrate specificity and selectivity observed in yeast compared with fatty acid profiles observed in canola events expressing these enzymes

The engineered biosynthetic pathway described in Examples 10-18 can convert oleic acid (18:1n-9), ALA (18:3n-3), and SDA (18:4n-3) to 20:5n-3 and 22:6n-3. The conversion of ALA and SDA to 20:5n-3 and 22:6n-3 is accomplished by the enzymes encoded in the engineered pathway and results from their ability to accept several substrates. The substrate tolerance of these enzymes cannot be predicted, but instead must be determined empirically as described in Examples 21 and 22. The ability of the desaturases and elongases, described in Examples 10-18, to use alternate substrates also provides a proof-reading mechanism within the engineered pathway that allows re-routing of undesired side-products back into the biosynthesis of 20:5n-3 and 22:6n-3.

Delta-6-desaturase:

Yeast feeding studies (Table 159 and Table 163) show that the delta-6-desaturase (*Ostreococcus tauri*) can accept two substrates, LA (18:2n-6) and ALA (18:3n-3), but prefers ALA slightly. The range of acceptable substrates (Tables 148-152) utilized by the specific combination of elongases and desaturases that were engineered into canola (Examples 10-18) allow the ALA desaturated by the delta-6-desaturase (*Ostreococcus tauri*) to ultimately be converted to DHA. The fatty acid analysis data presented for the engineered canola lines in Examples (10-18) show significantly lower levels of ALA relative to non-transgenic Kumily controls, consistent with the conversion of ALA to 20:5n-3 and 22:6n-3.

Delta-6-elongases:

Yeast feeding studies (Table 160 and Table 163) show that both of the delta-6-elongase's, *Physcomitrella patens* and *Thalassiosira pseudonana*, accept 18-carbon chain substrates but prefer molecules desaturated at carbon-6 (see GLA (18:3n-6) and SDA (18:4n-3) vs. LA (18:2n-6) and ALA (18:3n-3). The delta-6-elongase from *Thalassiosira pseudonana* is much more stringent than the one from *Physcomitrella patens* in recognizing the C-6 desaturation. In the fatty acid analysis of the engineered canola lines presented in Examples (10-18) 20:2n-6 and 20:3n-3, resulting from desaturation of LA and ALA respectively, are detected. In the LTM593 (Example 18) line the levels of 20:2n-6 and 20:3n-3 are about the same as found in wild-type Kumily.

Delta-5-desaturase:

Yeast feeding studies (Table 159 and Table 162 and Table 163) show that the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) accepts DHGLA (20:3n-6) and 20:4n-3. The ability of the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) to desaturate 20:4n-3 is important for the conversion of ALA and SDA into 20:5n-3 and 22:6n-3.

Omega-3-desaturases:

Yeast feeding show that both omega-3-desaturase's, (*Phythophthora infestans* and *Pythium irregulare*) can accept ARA (20:4n-6), DTA (22:4n-6), and. DHGLA (20:3n-6). The omega-3-desaturases prefer molecules with 4- double bonds. In the transgenic canola lines described in Examples (10-18), the ability of these omega-3-desaturases to utilize 22:4n-6 as a substrate allows re-direction of ARA (20:4n-6) that has been elongated by the delta-5-elongase (*Ostreococcus tauri*) back into the pathway for synthesis of 22:6n-3.

Delta-5-elongase:

Yeast feeding show (Table 161 and Table 163) that the delta-5-elongase (*Ostreococcus tauri*) prefers 20-carbon chain substrates, EPA (20:5n-3) and ARA (20:4n-6), but can also extend lipids containing 18-carbon chains, like SDA (18:4n-3) and ALA (18:3n-3). In the engineered canola (Examples 10-18) the presence of the omega-3-desaturase's, (*Phythophthora infestans* and *Pythium irregulare*), allows the 22:4n-6 product, resulting from the elongation of ARA, to be converted to 22:5n-3 and which is then desaturated by the delta-4-desaturases (*Thraustochytrium* sp.and *Pavlova lutheri*) to produce the final product, 22:6n-3. Fatty acid analysis of the transgenic canola lines described in Examples 10-18 shows minor levels of 22:4n-6, likely due to the ability of the omega-3 desaturases to redirect this lipid back into the biosynthesis of 22:6n-6. The 20:4n-3 produced from the extension of SDA is substrate recognized by the delta-5-desaturase (*Thraustochytrium* sp. ATCC21685) and can be converted to 20:5n-3, the preferred substrate of the delta-5-elongase (*Ostreococcus tauri*).

Delta-4-desaturases:

Yeast feeding studies (Table 159 and Table 163) show that both delta-4-desaturase's, (*Thraustochytrium* sp.and *Pavlova lutheri*) can accept 22:5n-3 and DTA (22:4n-6) with a slight preference for 22:5n-3.

Example 27: Using differentially labeled heavy peptides to monitor digestion efficiency in quantitative LC-MS.

Introduction:

The described method provides an avenue for quantitating digestion efficiency in targeted proteomics experiments. In most MRM LC-MS applications, measuring digestion efficiency relies on an indirect measurement which examines a peptide profile of the sample by comparing the number of missed cleavage peptides detected to the number of completely digested peptides detected in a separate LC-MS experiment. While providing an overall perspective of the trypsin efficiency within the experiment, the data does little to address specific digestion of the target sequence being monitored in the MRM experiment. Measuring global digestion efficiency does not address possible sequence specific biases in trypsin activity and may result in misrepresentation of target quantity. Indeed, there was a growing appreciation that trypsin digestion kinetics was strongly influenced by sequence context (1, 2). In order to measure sequence specific digestion efficiency we use two differentially-labeled heavy peptides to obtain a ratio for digestion efficiency (scheme 1). Peptide three includes the correct sequence context for the target by including four amino acids both amino and carboxyl to the two trypsin cleavage sites that generate the native peptide. In general, three amino acids were considered sufficient for trypsin to identify its cleavage site (3) and hydrolysis rates were only affected by the closest two amino acids (2). This scheme allows us to simultaneously assess specific digestion efficiency and also obtain quantitation data for the target molecule. In this design, the two peptides were spiked at equimolar concentrations into the test sample prior to digestion. During sample analysis, transitions were monitored for the target peptide and the two heavy peptide products. Peak intensities obtained from the two heavy peptides were compared to get a ratio for digestion efficiency and the target peptide intensities were used for quantitation.

Scheme 1

Example protein: O3D (Pir)

Sequence:

MASTSAAQDAAPYEFPSLTEIKRALPSECFEASVPLSLYYTARSLALAGSLAVALSYARALPLVQ

ANALLDATLCTGYVLLQGIVFWGFFTVGHDCGHGAFSRSHVLNFSVGTLMHSIILTPFESWKLS
HRHHHKNTGNIDKDEIFYPQREADSHPVSRHLVMSLGSAWFAYLFAGFPPRTMNHFNPWEAM
YVRRVAAVIISLGVLFAFAGLYSYLTFVLGFTTMAIYYFGPLFIFATMLVVTTFLHHNDEETPWYA
DSEWTYVKGNLSSVDRSYGALIDNLSHNIGTHQIHHLFPIIPHYKLNDATAAFAKAFPELVRKNA
APIIPTFFRMAAMYAKYGVVDTDAKTFTLKEAKAAAKTKSS

target peptide (underlined), native sequence contained in synthetic peptide for digestion efficiency (redouble underlined)

Peptides

DEIFYPQR (native peptide measured from sample)

DEIFYPQ($^{15}N^{13}C$R) (for standard curve, and normalization)

NIDKDEIFY($^{15}N^{13}C$P)Q($^{15}N^{13}C$R)EADS (for digestion efficiency)

Standard peptides (2) (3) were spiked into the test sample at the same concentration prior to proteolysis. After proteolysis the transitions for the product peptides, (2) and DEIFY(15N13CP)Q(15N13CR) were compared in product ion intensity in order to determine the digestion efficiency of the targeted sequence within the context of its native sequence according to the following equation:

$$\%\ digestion\ efficiency = \frac{\text{peak intensity DEIFY}(^{15}N^{13}C\text{P})\text{Q}(^{15}N^{13}C\text{R})}{\text{peak intensity DEIFYPQ}(^{15}N^{13}C\text{R})} * 100$$

The % digestion efficiency is therefore measured in the test sample directly and can be used to assess the impact on detected target concentration.

1) Proc J. et al. 2010. Journal of Proteome Res. 9:5422-5437.

2) Lowenthal M. et al. 2014. Anal Chem. 86:551-558.

3) Makriyannis T. and Y. Clonis. 1997. Biotech Bioeng. 53:49-57.

Example 28: Use of different germplasm to optimize production of VLC-PUFA

It appears that expression of the same or very similar gene sets in different host plants, such as *Camelina sativa* (Ruiz-Lopez et al., 2014), results in a larger yield of VLC-PUFA than has been attainable in *Brassica napus*. This notion is illustrated by the data in Table 179. Table 179 contains fatty acid data from seeds of transgenic *B. napus* (LBJ1671) and transgenic *C. sativa* (RRes_EPA_line) transformed with nearly identical T-DNAs and the respective wild type controls. The only difference between the constructs used to make the two transgenic lines is that the TDNA used to make the transgenic *B. napus* (LBJ1671) has an extra GFP expression cassette adjacent to the right border. When comparing transgenic *B. napus* (LJB1671) to the WT Kumily control, there is a decrease in the amount of 18:1 and increases in the amounts of 18:2 and

18:3 fatty acids. The 18:2 and 18:3 fatty acids are intermediates of the pathway introduced by LJB1671, and therefore the elongation of 18:2 and 18:3 fatty acids into 20C fatty acids can be considered a bottleneck reaction in *B. napus.* On the other hand, when comparing transgenic *C. sativa* (RRes_EPA_line) to the WT control, there is a decrease in 18:1 and also in 18:2 and 18:3 fatty acids. Therefore, the elongation of 18:2 and 18:3 fatty acids is not a bottleneck in *C. sativa.* The absence of this bottleneck in *C. sativa* results in 10-fold higher 20:5n-3 (EPA) content compared to the transgenic *B. napus.*

The conversion of 18:1 to 18:2 and of 18:2 to 18:3 occurs largely in the phospholipid membrane, while the elongation of 18C fatty acids to 20C fatty acids occurs strictly in the acyl-CoA pool. This difference in substrate specificity means that 18C fatty acids must be released from membrane phospholipids in order to be elongated. This release is apparently a bottleneck in *B. napus*, but not in *C. sativa*. The difference in flux of 18:2 from PC into the CoA pool between these two species could be due to differences in the activities of numerous enzymes, including: 1) phospholipases, 2) acyl-CoA synthetases, 3) lyso-PC acyltransferases, 4) phospholipid-diacylglycerol choline transferases, 5) phospholipid-diacylglycerol acyl transferases, and others. As a consequence of these observations, transforming any of the constructs from example 11 to example 18 into variety of species and germplasms (such as other Brassica germplasms in the so called Triangle of U), would result in a prefered embodiement of the present invention (e.g. higher amounts of EPA and DHA, and/or lower amounts of 18:2n-6).

Table 179: Introduction of a construct that was isogenic for all genes involved in VLC-PUFA synthesis into *Brassica napus* cv Kumily and into *Camelina sativa*.Average fatty acid data of segregating T1 seed of 26 events containing the T-DNA of LJB1671 and wild type (WT) *Brassica napus* cv Kumily are shown in the top two rows of data. For line RRes_EPA, fatty acid profiles of T3 homozygous seeds of selected insertion events, and of the respective WT control, are shown. RRes_EPA line and WT *Camelina sativa* data were taken from supplemental table S1 of Ruiz-Lopez *et al.*, 2014. n.d, not determined.

Event	Other	16:0	16:1 n-7	16:3 n-3	18:0	18:1 n-9	18:2 n-6	18:2 n-9	18:3 n-3	18:3 n-6	18:4 n-3	20:0	20:1 n-9	20:2 n-6	20:3 n-3	20:3 n-6	20:4 n-3	20:4 n-6	20:5 n-3	22:0	22:1 n-9
LJB1671 introduced into Kumily (n=26)	0	5 ± 0.5	0.2 ± 0.1	0.1 ± 0.1	2.6 ± 0.6	37.8 ± 10.9	29.1 ± 4.3	0.4 ± 0.4	7.8 ± 1.2	4.4 ± 5.4	2 ± 2.7	0.9 ± 0.2	1.3 ± 0.4	1.8 ± 1.8	0.6 ± 0.6	1.5 ± 1	1 ± 0.7	0.8 ± 0.5	2.1 ± 1.5	0.5 ± 0.1	0 ± 0
WT Kumily greenhouse (n=46)	0	5 ± 0.1	0.4 ± 0	0.2 ± 0	2.6 ± 0.1	66.3 ± 1.7	16.8 ± 1.3	0 ± 0	6.1 ± 0.4	0	0	0.9 ± 0	1.2 ± 0	0	0	0	0	0	0	0.5 ± 0	0 ± 0
RRes_EPA line	11.9 ± 1.0	8.3 ± 0.6	n.d	n.d	6.7 ± 0.9	5.0 ± 0.8	20.6 ± 1.2	n.d	15.8 ± 2.9	2.4 ± 1.7	1.5 ± 1.2	n.d	7.0 ± 0.9	n.d	n.d	1.1 ± 0.4	3.2 ± 1.1	1.6 ± 0.3	14.7 ± 2.8	n.d	n.d
WT *Camelina sativa*	11.2 ± 0.1	6.5 ± 0.1	n.d	n.d	3.4 ± 0.1	14.5 ± 0.4	19.0 ± 0.6	n.d	30.8 ± 0.6	0	0	n.d	14.5 ± 0.1	n.d	n.d	0	0	0	0	n.d	n.d

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Example 29: Fatty acid analysis of canola oils and commercial oil and solid samples (Total FAME analysis)

Canola seeds:
10-15 canola seeds were transferred into tubes on a 96-format rack and closed with cap strips. Seeds were ground in a swing mill using 3 mm beads for 2 x 2 min at 30 Hz. The rack was then centrifuged for 5 min at 4000 rpm to remove powder from the lid. Extraction of oil was carried out by adding 800 µL of methyl *tert*-butyl ether (MTBE) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 40 µL of the clear supernatant was transferred into a 96-well micro rack and diluted using 260 µL MTBE. Lipids were derivatized into fatty acid methyl esters (FAMEs) by adding 20 µL trimethylsulfonium hydroxide solution (TMSH, 0.2 M in methanol) into each sample. The rack was closed using silicone/PTFE cap mats and incubated for 20 min at room temperature.

Arabidopsis seeds:
20 mg of seeds were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

Canola oils and commercial oil samples:
20 µL of oil was transferred into a glass vial and diluted using 1 mL MTBE. 20-80 µL of the sample-solutions were transferred into into a 96-well micro rack and diluted using 260 µL MTBE. Lipids were derivatized into FAMEs by adding 20 µL trimethylsulfonium hydroxide solution (TMSH, 0.2 M in methanol) into each sample. The rack was closed using silicone/PTFE cap mats and incubated for 20 min at room temperature.

Commercial solid samples:
Solid samples were lyophilized prior to analysis. 20 mg of solid samples were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

An overview on the samples is provided in the following Table.

Table 180: Samples description

Sample Name	Description	Source/Origin
	Transgenic plant samples (T-DNA identity)	
LANBCH oil	*B. napus* (VC-LJB2197-1qcz, VC-LLM337-1qcz rc)	2014 Field season
LANPMZ seeds	*B. napus* (VC-LJB2197-1qcz, VC-LLM337-1qcz rc)	2012 Field season
LAODDN seeds	*B. napus* (VC-LJB2755-2qcz rc, VC-LLM391-2qcz rc)	2012 Field season
LBFDAU oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFGKN seeds	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFGKN oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFLFK seeds	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
LBFLFK oil	*B. napus* (VC-LTM593-1qcz rc)	2014 Field season
Arabidopsis seeds	*A. thaliana* (VC-LTM593-1qcz rc)	Greenhouse
	Non-transgenic plant samples	
Kumily seeds	wild type *B. napus*	2012 Field season
Kumily oil	wild type *B. napus*	2012 Field season
Canola Commodity oil	canola oil blend	
Clear Valley 65 oil	canola oil blend (65% oleic acid)	
Clear Valley 80 oil	canola oil blend (80% oleic acid)	
Brassica juncea seeds	wild type *B. juncea* (high erucic acid variety)	
Borage oil	oil preparation from plants (*Borago officinalis*)	
Echium oil	oil preparation from plants (*Echium plantagineum*)	
Primrose oil	oil preparation from plants (*Oenothera*)	
	Land animal fat samples	
Beef (corned beef)	prepared beef product	
Butter 1	butter from cow milk	
Butter 2	butter from cow milk	
Egg yolk from chicken	egg yolk powder from chicken	
	Fish/Crustacean oil samples	
Menhaden oil	oil preparation from fish (Menhaden)	
Salmon oil capsule	oil preparation from fish (Salmon)	
Tuna 1 oil	oil preparation from fish (Tuna)	
Anchovy 2 oil	oil preparation from fish (Anchovy)	
Krill oil capsule	oil preparation from crustacean (Krill)	
	Algal oil samples	
DHA DHA oil capsule	oil preparation from algae	
Omega EPA/DHA oil capsule	oil preparation from algae	
Omega-3 EPA & DHA	oil preparation from algae	
Omega 3 algae oil	oil preparation from algae	
	Fungal oil sample	
Mortierella alpina ARA-rich oil	oil preparation from fungi	

GC analysis:

An Agilent 7890A gas chromatograph coupled to Agilent flame ionization detector was used for FAME analysis. Separation of FAMEs was carried out on a DB-225 capillary column (20 m x 180 µm x 0.2 µm, Agilent) using H_2 as carrier gas with a flow rate of 0.8mL/min. The GC was operated in split mode using a split ratio of 1:50 at an injector temperature of 250 °C, injection volume was 1 µL. Oven temperature was held at 190 °C for 3 min and increased to 220 °C with 15 °C min^{-1}. Temperature was held at

220 °C for another 6 min. Peak detection and integration was carried out using Agilent GC ChemStation software (Rev. B.04.02 SP1).

Table 181: Fatty acids determined by FAME analysis

Shorthand Name	Trivial Name	Fatty Acid Name (systematic name)	Fatty Acid Souce
14:0	Myristic acid	Tetradecanoic acid	Supelco 37 Component FAME Mix
16:0	Palmitic acid	Hexadecanoic acid	Supelco 37 Component FAME Mix
16:1n-9		(Z)-7-Hexadecenoic acid	single standard
16:1n-7	Palmitoleic acid	(Z)-9-Hexadecenoic acid	Supelco 37 Component FAME Mix
17:0	Margaric acid	Heptadecanoic Acid	Supelco 37 Component FAME Mix
16:3n-3		(Z,Z,Z)-7,10,13-Hexadecatrienoic acid	single standard
18:0	Stearic acid	Octadecanoic acid	Supelco 37 Component FAME Mix
18:1n-9	Oleic acid	(Z)-9-Octadecenoic acid	Supelco 37 Component FAME Mix
18:1n-7	Vaccenic acid	(Z)-11-Octadecenoic acid	single standard
18:2n-9		(Z,Z)-6,9-Octadecadienoic acid	single standard
18:2n-6	Linoleic acid	(Z,Z)-9,12-Octadecadienoic acid	Supelco 37 Component FAME Mix
18:3n-6	gamma-Linolenic acid	(Z,Z,Z)-6,9,12-Octadecatrienoic acid	Supelco 37 Component FAME Mix
18:3n-3	alpha-Linolenic acid	(Z,Z,Z)-9,12,15-Octadecatrienoic acid	Supelco 37 Component FAME Mix
18:4n-3	Stearidonic acid	(Z,Z,Z,Z)-6,9,12,15-Octadecatetraenoic acid	single standard
20:0	Arachidic acid	Eicosanoic acid	Supelco 37 Component FAME Mix
20:1n-9	Gondoic acid	(Z)-11-Eicosenoic acid	Supelco 37 Component FAME Mix
20:2n-9		(Z,Z)-8,11-Eicosadienoic acid	no standard
20:3n-9	Mead acid	(Z,Z,Z)-5,8,11-Eicosatrienoic acid	single standard
20:2n-6		(Z,Z)-11,14-Eicosadienoic acid	Supelco 37

			Component FAME Mix
20:3n-6	Dihomo-gamma-linolenic acid	(Z,Z,Z)-8,11,14-Eicosatrienoic acid	Supelco 37 Component FAME Mix
20:4n-6	Arachidonic acid	(Z,Z,Z,Z)-5,8,11,14-Eicosatetraenoic acid	Supelco 37 Component FAME Mix
20:3n-3		(Z,Z,Z)-11,14,17-Eicosatrienoic acid	Supelco 37 Component FAME Mix
20:4n-3		(Z,Z,Z,Z)-8,11,14,17-Eicosatetraenoic acid	single standard
20:5n-3	Timnodonic acid	(Z,Z,Z,Z,Z)-5,8,11,14,17-Eicosapentaenoic acid	Supelco 37 Component FAME Mix
22:0	Behenic acid	Docosanoic acid	Supelco 37 Component FAME Mix
22:1n-9	Erucic acid	(Z)-13-Docosenoic acid	Supelco 37 Component FAME Mix
22:2n-6		(Z,Z)-13,16-Docosadienoic acid	Supelco 37 Component FAME Mix
22:4n-6	Adrenic acid	(Z,Z,Z,Z)-7,10,13,16-Docosatetraenoic acid	single standard
22:5n-6	Osbond acid	(Z,Z,Z,Z,Z)-4,7,10,13,16-Docosapentaenoic acid	single standard
22:4n-3		(Z,Z,Z,Z)-10,13,16,19-Docosatetraenoic acid	no standard
22:5n-3	Clupanodonic acid	(Z,Z,Z,Z,Z)-7,10,13,16,19-Docosapentaenoic acid	single standard
22:6n-3		(Z,Z,Z,Z,Z,Z)-4,7,10,13,16,19-Docosahexaenoic acid	Supelco 37 Component FAME Mix
24:0	Lignoceric acid	Tetracosanoic Acid	Supelco 37 Component FAME Mix
24:1n-9	Nervonic acid	Tetracosenoic Acid	Supelco 37 Component FAME Mix

The commercially available Supelco® 37 Component FAME Mix was used to identify retention times of 22 from 34 target fatty acids, 10 further fatty acids were spiked into the mix or analyzed separately to obtain retention times. For two of the target FAs, no commercial standard was available.

Peak areas of the detected target fatty acids were added up to yield the total peak area. The fatty acid profile and percentage of individual fatty acids was then calculated as peak area percent of total peak area.

The results are shown in the table 182.

Table 182 (Part A): Fatty acid profile of 34 target fatty acids from total lipids after MTBE extraction, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area.

Sample name	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	0,1	4,6	0,0	0,2	0,1	0,1	2,7	26,3	3,1	0,7	31,7	1,3	5,7	0,3	0,6	0,8
LANPMZ seeds	0,1	4,9	0,0	0,2	0,1	0,1	2,7	29,1	2,9	0,5	33,6	0,8	4,3	0,1	0,8	0,9
LAODDN seeds	0,1	5,2	0,0	0,2	0,1	0,2	2,5	28,2	3,2	0,6	35,1	1,4	5,6	0,4	0,8	0,7
LBFDAU oil	0,1	4,6	0,0	0,2	0,0	0,1	2,7	26,9	3,1	1,0	29,5	1,6	5,8	0,3	0,6	0,7
LBFGKN seeds	0,1	4,5	0,0	0,2	0,1	0,1	2,5	30,9	3,7	0,5	33,2	0,9	6,8	0,2	0,6	0,8
LBFGKN oil	0,1	4,4	0,0	0,2	0,0	0,1	2,6	32,2	2,9	0,5	33,2	0,9	6,8	0,2	0,6	0,8
LBFLFK seeds	0,1	4,8	0,0	0,2	0,1	0,1	2,7	26,4	3,9	0,9	30,3	1,6	5,7	0,3	0,6	0,8
LBFLFK oil	0,1	4,6	0,0	0,2	0,1	0,1	2,7	27,9	3,0	0,9	30,6	1,5	5,8	0,3	0,6	0,8
Arabidopsis seeds	0,1	7,0	0,0	0,2	0,1	0,1	5,3	8,9	1,8	0,2	22,3	0,3	16,8	0,1	3,5	17,5
Kumily seeds	0,1	4,7	0,0	0,3	0,1	0,1	1,9	57,1	3,8	0,0	20,1	0,0	9,3	0,0	0,6	1,1
Bovine fat (corned beef)	3,7	24,6	0,3	3,7	1,2	0,0	16,9	43,9	1,9	0,5	1,6	0,2	0,7	0,1	0,1	0,2
Butter 1	14,2	37,7	0,3	1,9	0,7	0,0	12,7	26,8	1,4	0,8	2,0	0,0	0,6	0,0	0,2	0,1
Butter 2	13,4	37,4	0,3	2,0	0,7	0,0	12,3	27,5	1,6	0,8	2,4	0,0	0,5	0,0	0,2	0,1
Egg yolk from chicken	0,5	25,0	0,6	2,2	0,3	0,0	8,7	35,7	1,5	0,1	19,1	0,3	0,8	0,2	0,1	0,3
Menhaden oil	8,9	17,1	0,2	12,6	2,3	0,1	3,0	7,2	3,4	0,1	1,8	0,4	1,5	3,4	0,3	1,1
Salmon oil capsule	7,8	17,0	0,3	8,6	1,7	0,1	3,5	9,4	3,4	0,2	1,9	0,4	1,1	2,8	0,6	1,9

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Tuna 1 0525 oil	3,9	19,7	0,4	5,0	1,3	0,0	5,1	14,9	2,5	0,1	2,0	0,3	0,5	1,0	0,4	1,5
Omevital Anchovy oil	7,3	18,0	0,3	8,6	1,8	0,1	3,9	9,6	3,2	0,2	1,8	0,5	0,9	3,3	0,6	1,6
Krill oil capsule	12,7	21,3	0,1	6,8	0,3	0,1	1,2	10,9	7,0	0,2	2,3	0,3	1,3	4,2	0,1	0,7
DHA DHA oil capsule	5,8	16,0	0,0	0,3	0,1	0,2	1,0	15,4	0,3	0,0	1,3	0,3	0,1	0,3	0,1	0,1
Omega EPA/DHA oil capsule	1,3	16,1	0,0	0,2	0,1	0,0	1,9	26,0	0,2	0,0	2,2	0,1	0,2	0,2	0,4	0,1
Omega-3 EPA & DHA	1,9	19,2	0,0	0,3	0,5	0,0	1,7	10,2	0,2	0,0	1,4	0,1	0,1	0,2	0,4	0,1
Omega 3 algae oil	5,4	15,4	0,0	0,3	0,1	0,2	1,0	15,0	0,2	0,0	1,5	0,3	0,1	0,4	0,1	0,1
Mortierella alpina ARA-rich oil	0,4	6,1	0,0	0,1	0,2	0,0	6,2	5,6	0,3	0,0	7,9	2,3	0,1	0,0	0,9	0,3

Table 182 (Part B): Fatty acid profile of 34 target fatty acids from total lipids after MTBE extraction, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area.

Sample name	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1
LANBCH oil	0,2	0,1	0,3	1,6	3,4	0,1	1,0	7,5	0,3	0,0	0,1	1,5	0,0	0,4	4,1	1,3	0,1	0,1
LANPMZ seeds	0,6	0,2	0,6	2,1	3,3	0,2	1,0	5,6	0,4	0,0	0,1	1,3	0,0	0,4	2,5	1,0	0,2	0,2
LAODDN seeds	0,1	0,1	0,1	1,5	1,2	0,1	1,0	6,0	0,4	0,0	0,0	1,1	0,0	0,5	2,7	0,9	0,2	0,2
LBFDAU oil	0,3	0,1	0,1	2,9	2,2	0,1	1,9	10,1	0,3	0,0	0,0	0,4	0,1	0,2	2,6	1,4	0,1	0,1
LBFGKN seeds	0,2	0,1	0,2	1,8	2,0	0,1	1,1	5,7	0,3	0,0	0,0	0,4	0,0	0,2	2,0	0,9	0,1	0,2
LBFGKN oil	0,2	0,1	0,2	1,7	1,9	0,1	1,0	5,5	0,3	0,0	0,0	0,4	0,0	0,1	1,9	0,9	0,1	0,1
LBFLFK seeds	0,2	0,1	0,1	2,8	2,2	0,1	1,6	8,4	0,3	0,0	0,0	0,7	0,1	0,4	3,3	1,3	0,1	0,2
LBFLFK oil	0,2	0,1	0,1	3,1	2,1	0,1	1,8	7,9	0,3	0,0	0,0	0,6	0,1	0,4	3,0	1,2	0,1	0,1
Arabidopsis seeds	0,6	0,6	1,4	0,3	0,5	1,7	0,5	5,6	0,5	2,0	0,0	0,7	0,0	0,0	0,1	1,3	0,2	0,4
Kumily seeds	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,1	0,2
Bovine fat (corned beef)	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0	0,0
Butter 1	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,1	0,1	0,0	0,0	0,2	0,0	0,0	0,1	0,0	0,0	0,0

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Butter 2	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,1	0,1	0,0	0,0	0,1	0,0	0,0	0,1	0,0	0,0	0,0
Egg yolk from chicken	0,0	0,1	0,2	0,2	1,9	0,0	0,0	0,4	0,0	0,0	0,0	0,1	0,4	0,0	0,1	1,3	0,0	0,0
Menhaden oil	0,2	0,0	0,3	0,3	1,3	0,3	1,7	16,4	0,2	0,2	0,0	0,3	0,6	0,1	2,8	12,1	0,2	0,3
Salmon oil capsule	0,2	0,0	0,2	0,2	1,6	0,1	1,0	19,5	0,2	0,3	0,0	0,2	0,4	0,1	2,5	12,7	0,1	0,6
Tuna 1 oil	0,0	0,0	0,3	0,2	2,2	0,2	0,5	7,5	0,2	0,2	0,0	0,3	1,8	0,1	1,3	26,6	0,2	0,6
Anchovy 2 oil	0,2	0,0	0,2	0,2	1,4	0,1	1,0	19,5	0,2	0,3	0,0	0,2	0,4	0,1	2,1	12,7	0,1	0,5
Krill oil capsule	0,0	0,0	0,1	0,2	0,8	0,1	0,5	18,4	0,1	0,5	0,0	0,0	0,2	0,0	0,4	9,2	0,1	0,1
DHA DHA oil capsule	0,0	0,0	0,0	0,8	0,7	0,1	0,8	1,1	0,2	0,0	0,0	0,1	15,6	0,1	0,4	38,6	0,5	0,0
Omega EPA/DHA oil capsule	0,0	0,0	0,0	0,2	1,4	0,1	0,6	17,0	0,3	0,0	0,0	0,2	1,2	0,0	3,1	26,8	0,2	0,0
Omega-3 EPA & DHA	0,0	0,0	0,0	0,3	1,8	0,1	0,8	19,5	0,2	0,1	0,0	0,3	1,9	0,0	3,3	35,6	0,1	0,0
Omega 3 algae oil	0,0	0,0	0,0	1,1	1,0	0,1	0,9	1,3	0,3	0,0	0,0	0,1	16,0	0,1	0,6	38,2	0,5	0,0
Mortierella alpina ARA-rich oil	0,0	0,0	0,4	4,4	48,5	0,5	0,0	0,2	3,4	0,1	0,0	0,5	0,0	0,2	0,0	0,1	11,2	0,4

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Example 30: Lipid Profiling of canola oils and commercial oil samples:

Canola seeds:

12-15 canola seeds were transferred into micro tubes on a 96-format rack and closed with cap strips. Seeds were ground in a swing mill using 3 mm beads for 2 x 2 min at 30 Hz. The rack was then centrifuged for 5 min at 4000 rpm to remove powder from the lid. Extraction of oil was carried out by adding 800 µL of dichloromethane/methanol (DCM/MeOH, 2:1 *v/v*) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 100 µL of the clear supernatant was transferred into glass vials and dried to remove the solvent.

Arabidopsis seeds:
20 mg of seeds were transferred into tubes on a 96-format rack. The canola seed protocol was followed in all further steps.

Canola oils and commercial oil samples:
200 µL of oil was transferred into into micro tubes on a 96-format rack and closed with cap strips. Extraction of oil was carried out by adding 800 µL of dichloromethane/methanol (DCM/MeOH, 2:1 *v/v*) to the samples followed by extraction in a swing mill for 2 x 30 sec at 30 Hz. After centrifugation at 4000 rpm for 10 min, 100 µL of the clear supernatant was transferred into glass vials and dried to remove the solvent.

Normal Phase Fractionation:

The dry residues were resuspended in 100 µL 2,2,4-trimethylpentane and isopropyl alcohol (TMP/IPA (9:1, v/v)) and vortexed thoroughly. The krill oil sample was further diluted 1:30 using TMP/IPA. For triacylglycerol (TAG) isolation, the oil samples were resuspended in 500 µL TMP/IPA prior to fractionation Separation of lipid classes was performed using normal phase HPLC that leads to interaction of lipids and stationary phase based on lipid species (e.g. head group in case of phospholipids) rather than length of fatty acid chain. An Agilent 1200 series HPLC was used that comprised

G1322A degasser, G1311A quaternary pump, G1310A isocratic pump, G1316A thermostatted column compartment (TCC), G1367B HiP autosampler, G1330B FC/ALS Thermostat and G1364 Fraction Collector (all Agilent) coupled to and Alltech 3300 evaporative light scattering detector (ELSD, Grace). A quaternary gradient from TMP, MTBE, acetonitrile (MeCN), DCM, formic acid (FA), ammonium formiate (0.5 M) and water was used for separation of lipid species on a PVA-Sil column (150 mm x 3 mm x 5 µm, YMC) with an initial flow rate of 0.4 mL/min. The injection volume was 50 µL. Fractions of triacylglycerol (TAG), diacylglycerol (DAG), monoacylglycerol (MAG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) were collected in glass vials. The solvent was removed and the residue was resuspended in 100 µL toluene. Derivatization of lipid species into FAMEs for subsequent fatty acid profiling was carried out using 20 µL TMSH. FAME analysis was performed as described previously for Example 29 with an injection volume of 2 µL.

The results are shown in the tables 183 to 187.

Table 183 (Part A): Fatty acid profile of 34 target fatty acids from monoacylglycerol (MAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	MAG	0,0	6,6	0,0	0,5	0,0	0,2	3,1	24,5	7,6	0,5	30,3	1,2	4,4	0,3	0,7	0,7
LANPMZ seeds	MAG	0,0	10,6	0,0	1,0	0,0	0,5	4,7	23,2	17,6	0,0	26,1	0,6	2,8	0,0	0,5	0,0
LAODDN seeds	MAG	0,4	12,1	0,0	1,1	0,0	0,0	6,4	22,2	15,5	0,0	25,3	1,3	4,0	0,0	1,1	0,0
LBFDAU oil	MAG	0,3	6,2	0,0	0,6	0,0	0,0	2,8	24,6	7,7	0,8	28,7	1,5	5,0	0,3	0,6	0,7
LBFGKN oil	MAG	0,3	6,8	0,0	0,6	0,0	0,0	3,2	28,1	7,6	0,4	28,6	0,8	4,9	0,0	0,7	0,7
LBFLFK seeds	MAG	0,0	12,7	0,0	0,9	0,0	0,0	6,8	22,8	13,2	0,3	23,7	1,1	4,3	0,0	0,9	0,0
LBFLFK oil	MAG	0,2	6,7	0,0	0,6	0,0	0,2	3,0	25,3	8,2	0,7	28,9	1,4	4,7	0,2	0,6	0,7

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Kumily oil	MAG	0,0	7,4	0,0	0,0	0,0	1,9	3,6	51,0	4,5	0,0	19,7	0,0	9,4	0,0	0,0	2,5
Canola Commodity oil	MAG	0,7	7,5	0,0	0,5	0,0	2,3	3,3	47,2	5,1	0,0	20,8	0,0	8,9	0,0	0,0	3,7
Clear Valley 65 oil	MAG	0,5	13,8	0,0	2,8	0,0	1,4	2,6	28,9	31,7	0,0	14,2	0,0	0,7	0,0	0,7	2,0
Clear Valley 80 oil	MAG	0,0	5,7	0,0	0,6	0,0	3,1	3,4	65,0	5,4	0,0	9,8	0,0	3,1	0,0	0,0	4,0
Menhaden oil	MAG	10,4	24,1	0,0	12,5	2,2	0,1	5,2	6,2	3,8	0,0	1,5	0,6	1,4	3,1	0,4	0,5
Salmon oil capsule	MAG	8,2	22,1	0,0	8,8	1,8	0,5	6,3	8,7	3,9	0,0	1,5	0,0	0,8	2,5	0,8	1,1
Krill oil capsule	MAG	5,4	21,0	0,0	3,4	0,0	1,8	3,3	7,2	6,4	0,0	1,9	0,0	0,0	2,6	0,0	1,9
DHA DHA oil capsule	MAG	7,8	24,4	0,0	1,0	0,0	0,0	3,1	10,1	0,0	0,0	3,5	0,0	0,0	0,0	0,0	0,0
Omega EPA/DHA oil capsule	MAG	2,2	25,8	0,0	0,0	0,0	0,0	3,3	16,0	0,0	0,0	3,2	0,0	0,0	0,0	0,0	0,0
Omega 3 algae oil	MAG	5,4	29,9	0,0	0,0	0,0	0,0	3,0	6,2	0,0	0,0	2,7	0,0	0,0	0,0	0,0	0,8

Table 183 (part B): Fatty acid profile of 34 target fatty acids from monoacylglycerol (MAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

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sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6	20:4n-6	20:3n-3	20:4n-3	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3	22:6n-3	24:0	24:0
LANBCH oil	MAG	0,3	0,3	0,2	1,8	2,8	0,8	1,0	5,4	0,7	0,0	0,0	1,9	0,0	0,3	2,8	1,3	0,2	0,2
LANPMZ seeds	MAG	0,0	0,0	0,0	1,9	2,8	0,0	1,4	3,9	0,0	0,0	0,0	0,0	0,0	0,0	1,4	1,1	0,0	0,0
LAODDN seeds	MAG	0,0	0,0	0,0	1,0	1,0	0,0	1,3	4,1	0,0	0,0	0,0	0,0	0,0	0,0	1,6	1,5	0,0	0,0
LBFDAU oil	MAG	0,3	0,2	0,2	2,8	1,9	0,0	2,0	8,0	0,0	0,0	0,0	1,3	0,0	0,0	2,2	1,4	0,0	0,0
LBFGKN oil	MAG	0,0	0,3	0,2	1,7	1,7	0,7	1,0	3,9	0,0	0,0	0,0	4,9	0,0	0,4	1,5	1,0	0,3	0,3
LBFLFK seeds	MAG	0,0	0,0	0,0	2,4	1,1	0,0	1,0	4,6	0,0	0,0	0,0	0,7	0,0	0,4	2,0	1,3	0,0	0,0
LBFLFK oil	MAG	0,2	0,3	0,2	2,7	1,4	0,0	1,7	5,9	0,0	0,0	0,0	2,1	0,0	0,4	2,3	1,1	0,3	0,3
Kumily oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Canola Commodity oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 65 oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,7
Clear Valley 80 oil	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

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Menhaden oil	MAG	0,1	0,0	0,3	0,5	1,5	0,0	1,4	12,3	0,0	0,0	0,0	0,0	0,0	0,1	2,2	9,6	0,2	0,2
Salmon oil capsule	MAG	0,0	0,0	0,0	0,4	1,6	0,0	0,0	17,2	0,0	0,0	0,0	0,0	0,0	0,0	2,1	11,7	0,0	0,0
Krill oil capsule	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	27,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	17,4	0,0	0,0
DHA DHA oil capsule	MAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	12,1	0,0	0,0	38,1	0,0	0,0
Omega EPA/DHA oil capsule	MAG	0,0	0,0	0,0	0,0	1,4	0,0	0,0	17,6	0,0	0,0	0,0	0,0	0,0	0,0	3,0	27,6	0,0	0,0
Royal Green Omega 3 algae oil	MAG	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	14,3	0,0	0,0	36,7	0,0	0,0

Table 184 (part A): Fatty acid profile of 34 target fatty acids from diacylgylcerol (DAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9

LANBCH oil	DAG	0,0	5,4	0,0	0,0	0,0	0,0	2,6	22,0	5,0	0,8	32,7	1,4	5,8	0,0	0,0	0,0
LBFDAU oil	DAG	0,0	4,7	0,0	0,0	0,0	0,0	2,3	22,4	4,9	0,5	29,9	1,7	6,3	0,0	0,0	0,0
LBFGKN oil	DAG	0,0	5,9	0,0	0,0	0,0	0,0	2,6	25,8	4,9	0,0	33,2	1,1	7,2	0,0	0,0	0,0
LBFLFK oil	DAG	0,0	6,1	0,0	0,0	0,0	0,0	2,5	22,9	5,1	0,8	30,7	1,7	6,4	0,0	0,0	0,0
Canola Commodity oil	DAG	0,0	8,3	0,0	0,0	0,0	2,7	0,0	53,9	0,0	0,0	21,6	0,0	10,1	0,0	0,0	3,4
Clear Valley 65 oil	DAG	0,0	9,4	0,0	1,7	0,0	3,6	0,0	47,6	17,3	0,0	16,8	0,0	0,0	0,0	0,0	3,7
Clear Valley 80 oil	DAG	0,0	7,0	0,0	0,0	0,0	3,6	0,0	71,6	4,6	0,0	8,8	0,0	0,0	0,0	0,0	4,3
Menhaden oil	DAG	10,4	23,6	0,0	12,6	2,4	0,0	4,9	6,4	3,8	0,0	1,5	0,0	1,5	3,6	0,0	0,8
Salmon oil capsule	DAG	8,4	21,0	0,0	8,6	2,1	0,0	5,3	8,3	3,7	0,0	1,4	0,0	0,0	4,0	0,0	0,9
DHA DHA oil capsule	DAG	6,8	17,5	0,0	0,0	0,0	0,0	0,0	19,7	0,0	0,0	2,3	0,0	0,0	0,0	0,0	1,3
Omega EPA/DHA oil capsule	DAG	2,0	16,7	0,0	0,0	0,0	0,0	3,3	32,7	0,0	0,0	2,5	0,0	0,0	0,0	0,0	1,1
Omega 3 algae oil	DAG	5,0	20,7	0,0	0,0	0,0	0,0	3,0	11,1	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0

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Table 184 (part B): Fatty acid profile of 34 target fatty acids from diacylgylcerol (DAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANBCH oil	DAG	0,0	0,0	0,0	2,2	4,2	0,0	1,1	8,8	0,0	0,0	0,0	2,1	0,0	0,0	4,1	2,0	0,0	0,0
LBFDAU oil	DAG	0,0	0,0	0,0	3,2	2,8	0,0	2,4	11,7	0,0	0,0	0,0	2,1	0,0	0,0	2,7	2,5	0,0	0,0
LBFGKN oil	DAG	0,0	0,0	0,0	2,7	2,8	0,0	0,8	7,4	0,0	0,0	0,0	0,9	0,0	0,0	2,4	2,5	0,0	0,0
LBFLFK oil	DAG	0,0	0,0	0,0	3,5	2,7	0,0	2,2	9,3	0,0	0,0	0,0	1,4	0,0	0,0	3,2	1,7	0,0	0,0
Canola Commodity oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 65 oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Clear Valley 80 oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Menhaden oil	DAG	0,0	0,0	0,0	0,0	1,0	0,0	1,5	13,4	0,0	0,0	0,0	0,0	0,0	0,0	2,3	10,3	0,0	1,2
Salmon oil	DAG	0,0	0,0	0,0	0,0	2,4	0,0	0,0	19,6	0,0	0,0	0,0	0,0	0,0	0,0	1,9	12,3	0,0	0,0

capsule																		
DHA DHA oil capsule	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,4	0,0	0,0	42,0	0,0	0,0
Omega EPA/DHA oil capsule	DAG	0,0	0,0	0,0	0,0	2,3	0,0	0,0	13,6	0,0	0,0	0,0	0,0	0,0	0,0	1,8	24,0	0,0	0,0
Omega 3 algae oil	DAG	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,9	0,0	0,0	44,5	0,0	0,0

Table 185 (part A): Fatty acid profile of 34 target fatty acids from triacylgylcerol (TAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANBCH oil	TAG	0,1	4,5	0,0	0,2	0,1	0,1	2,8	26,2	3,1	0,7	31,5	1,3	5,6	0,2	0,7	0,8
LANPMZ seeds	TAG	0,1	4,9	0,0	0,1	0,1	0,1	2,8	27,9	2,4	0,5	34,9	0,9	4,0	0,1	0,8	0,9
LAODDN seeds	TAG	0,1	5,0	0,0	0,2	0,1	0,2	2,8	29,1	2,7	0,6	35,0	1,4	5,4	0,3	0,8	0,8
LBFDAU oil	TAG	0,1	4,5	0,1	0,2	0,1	0,1	2,8	26,9	3,0	1,0	29,1	1,6	5,8	0,3	0,7	0,8
LBFGKN oil	TAG	0,1	4,3	0,0	0,2	0,1	0,1	2,7	32,4	2,9	0,5	33,0	0,9	6,8	0,2	0,7	0,8
LBFLFK seeds	TAG	0,1	4,5	0,1	0,2	0,1	0,1	2,7	28,6	3,4	0,9	30,1	1,3	5,8	0,2	0,7	0,8

LBFLFK oil	TAG	0,1	4,5	0,0	0,2	0,1	0,1	2,8	28,0	2,9	0,9	30,3	1,5	5,7	0,2	0,7	0,8
Arabidopsis seeds	TAG	0,1	6,7	0,1	0,2	0,1	0,1	5,0	7,8	1,6	0,2	21,7	0,2	17,4	0,1	3,6	18,9
Kumily seeds	TAG	0,1	4,8	0,0	0,3	0,1	0,1	2,0	59,2	0,0	0,0	20,5	0,0	10,3	0,0	0,7	1,2
Canola Commodity oil	TAG	0,1	3,9	0,0	0,2	0,1	0,1	1,8	60,0	2,9	0,0	19,0	0,0	9,3	0,0	0,6	1,3
Clear Valley 65 oil	TAG	0,0	4,1	0,0	0,2	0,1	0,1	1,8	69,5	2,2	0,0	17,6	0,0	0,8	0,0	0,8	1,5
Clear Valley 80 oil	TAG	0,0	2,9	0,0	0,1	0,1	0,1	2,6	80,0	0,0	0,0	8,2	0,0	2,3	0,0	0,9	1,8
Brassica juncea seeds	TAG	0,1	2,8	0,0	0,2	0,0	0,1	1,0	7,0	1,2	0,0	15,2	0,0	12,8	0,0	0,8	4,7
Menhaden oil	TAG	8,7	17,0	0,0	12,8	2,3	0,1	3,1	7,0	3,5	0,1	1,7	0,4	1,5	3,7	0,0	1,1
Salmon oil capsule	TAG	7,5	16,8	0,0	8,7	1,7	0,1	3,7	9,2	3,5	0,1	1,6	0,2	0,9	3,0	0,6	2,0
Krill oil capsule	TAG	21,9	21,4	0,0	11,7	0,4	0,1	1,7	17,4	8,0	0,1	2,5	0,3	1,3	5,5	0,1	1,0
DHA DHA oil capsule	TAG	5,7	15,9	0,0	0,2	0,1	0,2	1,0	15,2	0,2	0,0	1,2	0,2	0,1	0,3	0,1	0,1
Omega EPA/DHA oil capsule	TAG	1,1	15,5	0,0	0,1	0,1	0,0	1,9	25,9	0,2	0,0	2,1	0,0	0,1	0,1	0,4	0,1
Omega 3 algae oil	TAG	5,1	14,7	0,0	0,2	0,1	0,2	1,0	14,8	0,2	0,0	1,2	0,3	0,1	0,3	0,1	0,1

Table 185 (part B): Fatty acid profile of 34 target fatty acids from triacylglycerol (TAG) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANBCH oil	TAG	0,2	0,1	0,3	1,7	3,4	0,1	1,0	7,6	0,3	0,0	0,1	1,5	0,0	0,4	4,3	1,3	0,1	0,1
LANPMZ seeds	TAG	0,5	0,2	0,5	2,2	3,5	0,2	1,0	5,7	0,4	0,0	0,1	1,3	0,0	0,4	2,4	1,0	0,2	0,2
LAODDN seeds	TAG	0,1	0,1	0,1	1,5	1,3	0,1	0,9	6,0	0,4	0,0	0,0	1,1	0,0	0,4	2,7	0,8	0,2	0,2
LBFDAU oil	TAG	0,3	0,1	0,1	3,0	2,3	0,1	1,9	10,3	0,3	0,0	0,0	0,3	0,0	0,2	2,7	1,4	0,1	0,1
LBFGKN oil	TAG	0,2	0,1	0,2	1,8	1,9	0,1	1,0	5,6	0,3	0,0	0,0	0,3	0,0	0,1	1,9	0,9	0,1	0,1
LBFLFK seeds	TAG	0,3	0,1	0,1	3,1	2,0	0,1	1,7	7,7	0,3	0,0	0,0	0,5	0,1	0,5	3,0	1,2	0,1	0,1
LBFLFK oil	TAG	0,3	0,1	0,1	3,2	2,1	0,1	1,8	8,0	0,3	0,0	0,0	0,5	0,1	0,4	3,1	1,2	0,1	0,1
Arabidopsi	TAG	0,7	0,6	1,5	0,2	0,3	1,8	0,5	6,0	0,5	2,3	0,1	0,2	0,2	0,0	0,0	1,2	0,2	0,4

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s seeds																			
Kumily seeds	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,2	0,2
Canola Commodity oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,2	0,2
Clear Valley 65 oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,6	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,4	0,2
Clear Valley 80 oil	TAG	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,4	0,2
Brassica juncea seeds	TAG	0,0	0,0	0,9	0,0	0,0	0,2	0,0	0,0	1,2	49,3	1,6	0,0	0,5	0,0	0,0	0,0	0,7	2,2
Menhaden oil	TAG	0,2	0,0	0,3	0,3	1,3	0,2	1,8	16,7	0,2	0,2	0,0	0,3	0,6	0,1	2,9	12,1	0,1	0,4
Salmon oil capsule	TAG	0,2	0,0	0,2	0,2	1,7	0,1	1,1	20,0	0,2	0,4	0,0	0,2	0,5	0,1	2,6	13,0	0,1	0,6
Krill oil capsule	TAG	0,0	0,0	0,1	0,0	0,2	0,1	0,3	3,8	0,0	0,5	0,0	0,0	0,0	0,0	0,2	1,7	0,0	0,0
DHA DHA	TAG	0,0	0,0	0,0	0,9	0,5	0,0	0,8	0,8	0,2	0,0	0,0	0,1	16,0	0,2	0,4	39,2	0,5	0,0

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oil capsule																		
Omega EPA/DHA oil capsule	TAG	0,0	0,0	0,0	0,3	1,4	0,1	0,6	17,1	0,3	0,0	0,0	0,2	1,2	0,1	3,3	27,4	0,4	0,0
Omega 3 algae oil	TAG	0,0	0,0	0,0	1,0	0,6	0,1	0,9	1,2	0,2	0,0	0,0	0,1	16,9	0,1	0,6	39,9	0,3	0,0

Table 186 (part A): Fatty acid profile of 34 target fatty acids from phosphatidylcholine (PC) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANPMZ seeds	PC	0,0	12,7	0,6	0,3	0,1	0,1	3,4	6,6	5,8	0,0	39,2	1,8	4,5	0,3	0,0	0,0
LAODDN seeds	PC	0,0	11,5	0,4	0,7	0,0	0,0	3,1	9,7	5,9	0,0	37,0	4,5	10,1	1,5	0,0	0,0
LBFLFK seeds	PC	0,0	8,7	0,4	0,5	0,0	0,3	2,6	10,8	7,6	0,0	39,3	1,3	6,4	0,3	0,2	0,2
Arabidopsis seeds	PC	0,0	13,2	0,9	0,7	0,0	0,0	6,6	4,1	5,2	0,0	27,9	1,2	14,4	0,6	0,9	6,8
Kumily seeds	PC	0,0	6,1	0,2	0,6	0,0	0,2	0,7	57,8	2,7	0,0	27,2	0,0	4,5	0,0	0,0	0,0
Brassica juncea seeds	PC	0,0	12,3	0,0	1,8	0,0	1,1	1,5	29,4	4,6	0,0	34,4	0,0	12,0	0,0	0,0	1,9

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Krill oil capsule	PC	3,1	26,5	0,0	1,7	0,1	0,0	1,0	4,6	5,5	0,2	2,1	0,3	1,5	3,0	0,0	0,3

Table 186 (part B): Fatty acid profile of 34 target fatty acids from phosphatidylcholine (PC) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6 (DGLA)	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANPMZ seeds	PC	0,0	0,0	0,0	0,7	0,0	0,3	7,0	7,3	0,0	0,0	0,0	0,0	0,0	0,0	0,4	9,0	0,0	0,0
LAODDN seeds	PC	0,0	0,0	0,0	0,2	0,0	0,0	6,8	3,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,3	0,0	0,0
LBFLFK seeds	PC	0,1	0,0	0,0	0,9	0,0	0,1	10,9	4,1	0,0	0,0	0,0	0,0	0,0	0,2	1,8	3,3	0,0	0,0
Arabidopsis seeds	PC	0,0	0,0	0,0	0,0	0,0	2,8	1,0	6,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,2	0,0	0,0
Kumily seeds	PC	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Brassica	PC	0,0	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

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juncea seeds																		
Krill oil capsule	PC	0,1	0,0	0,1	0,2	0,7	0,0	1,0	33,3	0,0	0,6	0,0	0,0	0,0	0,0	0,6	13,6	0,0	0,1

Table 187 (part A): Fatty acid profile of 34 target fatty acids from phosphatidylethanolamine (PE) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	14:0	16:0	16:1n-9	16:1n-7	17:0	16:3n-3	18:0	18:1n-9	18:1n-7	18:2n-9	18:2n-6 (LA)	18:3n-6 (GLA)	18:3n-3 (ALA)	18:4n-3 (SDA)	20:0	20:1n-9
LANPMZ seeds	PE	0,0	18,3	0,0	0,0	0,0	0,0	3,1	5,8	6,4	0,0	36,4	1,3	4,4	0,0	0,0	0,0
LAODDN seeds	PE	0,0	17,7	0,0	0,0	0,0	0,0	3,3	7,9	6,0	0,0	33,6	3,0	8,5	0,0	0,0	0,0
LBFLFK seeds	PE	0,0	15,5	0,0	0,0	0,0	0,0	2,9	8,6	7,3	0,0	37,6	1,2	6,1	0,0	0,0	0,0
Arabidopsis seeds	PE	0,0	18,7	0,4	0,0	0,0	0,0	5,6	2,4	4,6	0,0	27,1	0,0	13,2	0,0	0,0	4,1
Brassica juncea seeds	PE	0,0	19,1	0,0	1,1	0,0	2,1	0,0	13,4	4,5	0,0	41,4	0,0	13,3	0,0	0,0	4,1
Krill oil capsule	PE	1,1	16,6	0,0	0,0	0,0	2,9	0,0	4,1	13,6	0,0	0,0	0,0	0,0	0,0	0,0	4,0

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Table 187 (part B): Fatty acid profile of 34 target fatty acids from phosphatidylethanolamine (PE) fraction after DCM/MeOH (2:1) extraction, normal phase fractionation, derivatization to FAMES and analysis by GC-FID. Data are mean, n=2 and FA expressed as peak area % from total peak area

sample name	Fraction	20:2n-9	20:3n-9	20:2n-6	20:3n-6	20:4n-6 (ARA)	20:3n-3	20:4n-3 (ETA)	20:5n-3 (EPA)	22:0	22:1n-9	22:2n-6	22:4n-6	22:5n-6	22:4n-3	22:5n-3 (DPA)	22:6n-3 (DHA)	24:0	24:1n-9
LANPMZ seeds	PE	0,0	0,0	0,0	1,0	6,5	0,0	4,1	4,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,5	0,0	0,0
LAODDN seeds	PE	0,0	0,0	0,0	0,0	7,5	0,0	4,2	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,9	0,0	0,0
LBFLFK seeds	PE	0,0	0,0	0,0	1,2	3,6	0,0	8,1	3,3	0,0	0,0	0,0	0,0	0,0	0,0	0,9	3,6	0,0	0,0
Arabidopsis seeds	PE	0,0	0,0	0,0	0,0	11,0	0,5	0,0	4,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,7	0,0	0,0
Brassica juncea seeds	PE	0,0	0,0	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
Krill oil capsule	PE	0,0	0,0	0,0	0,0	2,5	0,0	0,0	24,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	30,4	0,0	0,0

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Example 31: Phospholipid species profiling

Diluted extracts from Example 30 were used for phospholipid species analysis

PC species profiling

Phosphatidylcholine (PC) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. PC species separation was carried out on a Phenomenex Kinetex C8 column (150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/sodium acetate (50 mM) as solvent A and MeOH/sodium acetate (50 mM) as solvent B with a flow rate of 0.5 mL/min. The initial conditions were 40% A followed by a linear gradient to 25% A in a total run time of 7 min. MS was operated in positive ESI mode with a source temperature of 550 °C using scheduled multiple reaction monitoring (sMRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+Na]^+$ from phosphatidylcholine species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 78 PC species. In sMRM-mode, the neutral loss of fatty acids was monitored. MRMs of PC species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list.

All peak areas of target PC species were added to a total peak area. Species with peak areas lower than 10.000 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. PC species profile was then calculated as PC species percent of total PC species.

LPC species profiling

Lysophosphatidylcholine (LPC) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. LPC species separation was carried out on a Phenomenex Kinetex C8 column

(150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/formic as solvent A and MeOH as solvent B with a flow rate of 0.5 mL/min and a linear gradient from 80% A to 0% A over a total run time of 4 min. MS was operated in positive ESI mode with a source temperature of 550 °C using multiple reaction monitoring (MRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+H]^+$ from LPC species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated. In sMRM-mode, the neutral loss of fatty acids was monitored.

All peak areas of target LPC species were added to a total peak area. Species with peak areas lower than 12.500 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. LPC species profile was then calculated as LPC species percent of total LPC species.

PE/LPE species profiling

Phosphatidylethanolamine (PE) and lysophosphatidylethanolamine (LPE) species separation was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. PE/LPE species separation was carried out on a Phenomenex Kinetex C8 column (150 mm x 2.1 mm x 1.7 µm). The mobile phase consisted of H_2O/MeOH/ammonium formate as solvent A and MeOH/ ammonium formate as solvent B with a flow rate of 0.5 mL/min. The initial conditions were 100% A followed by a linear gradient to 0% A over a total run time of 16 min. MS was operated in negative ESI mode with a source temperature of 550 °C using scheduled multiple reaction monitoring (sMRM) and an injection volume of 1 µL. Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M-H]^-$ from PE and LPE species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 78 PE species and 12 LPE species.

MRMs of PE/LPE species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list.

All peak areas of target PE species and LPE species were added to a total peak area separately. The peak area cut-off for LPE not included into the calculation due to poor signal-to-noise was 2000 counts, for PE it was 5000 counts. PE and LPE species profile were then calculated as PE species percent of total PE species and LPE species percent of total LPE species.

The results are shown in Tables 188 to 191.

Table 188: Phosphatidylcholin species profile of occurring PC species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by +ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 and expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
PC140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0
PC140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0
PC140 182	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0
PC140 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,0	0,0	0,0
PC140 204	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PC140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,5	0,0	0,0	0,0
PC140 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PC140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0	0,0	0,0
PC160 160	0,5	0,7	0,4	0,4	0,3	0,0	0,4	0,9	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PC160 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0
PC160 180	0,0	0,3	0,2	0,0	0,2	0,0	0,3	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC160 181	4,9	4,8	5,2	4,6	6,6	7,5	5,8	4,5	3,7	11,5	12,9	12,5	15,9	9,0	11,8	0,0	10,8	3,4	6,1	6,4
PC160 182	12,4	11,7	10,3	12,6	8,8	10,1	7,6	11,5	11,6	7,5	6,7	7,3	7,4	0,0	11,4	0,0	3,9	14,7	12,9	20,5
PC160 183	3,9	2,3	4,7	3,5	2,0	4,8	1,8	3,4	8,2	3,2	1,9	1,7	0,7	0,0	8,1	0,0	1,8	0,0	0,0	0,0
PC160 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PC160 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,0	0,0	0,0	0,0

PC160 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0
PC160 226	0,0	2,7	1,5	0,0	1,3	0,0	1,2	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,4	0,0	0,0	0,0
PC161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PC161 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC161 181	0,0	0,3	0,4	0,0	0,4	0,0	0,5	0,0	0,7	0,7	0,0	0,8	0,3	0,8	0,4	0,0	0,9	0,0	0,0	0,0
PC161 182	0,0	0,8	0,7	0,0	0,5	0,0	0,4	0,0	1,7	1,1	0,0	0,8	0,6	0,0	1,6	0,0	0,2	0,0	0,0	0,3
PC161 183	0,0	0,3	0,7	0,0	0,3	0,0	0,4	0,0	0,6	0,5	0,0	0,0	0,0	0,0	1,2	0,0	2,7	0,0	0,0	0,0
PC161 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PC161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,4	0,0	0,0	0,0
PC161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	0,0	0,0	0,0
PC180 181	0,0	0,5	0,6	0,0	1,4	0,0	1,2	0,0	0,8	1,8	0,0	0,8	0,8	0,0	1,7	0,0	0,2	0,0	0,0	1,2
PC180 182	3,6	5,2	4,3	3,7	3,8	0,0	4,0	2,9	6,6	0,2	0,2	0,0	0,1	0,0	0,8	0,0	0,3	3,2	3,3	6,1
PC180 183	0,0	1,0	1,8	0,0	0,7	0,0	0,8	0,0	4,1	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,3	0,0	0,0	0,0
PC180 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,2	0,0	0,0	0,0
PC180 226	0,0	0,8	0,6	0,0	0,4	0,0	0,3	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0
PC181 181	5,8	3,4	4,2	7,0	7,8	15,5	8,0	6,6	2,3	26,8	36,1	28,0	32,9	66,0	15,2	29,5	4,4	9,0	18,6	7,5
PC181 182	15,8	13,5	13,1	17,8	16,7	19,4	16,0	17,6	9,3	24,4	27,4	27,0	28,6	19,1	18,7	0,0	2,2	19,2	16,9	24,5
PC181 183	3,4	2,7	5,1	4,1	3,5	4,3	3,8	3,9	4,4	7,0	5,0	6,0	1,8	4,9	7,4	0,0	0,7	0,0	0,0	0,0
PC181 204	3,0	1,9	1,8	4,9	2,6	3,5	4,6	4,2	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0
PC181 205	0,0	1,2	0,7	0,0	1,2	0,0	1,3	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	7,5	0,0	0,0	0,0
PC181 226	0,0	1,4	1,0	0,0	1,1	0,0	1,2	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,1	0,0	0,0	0,0
PC182 182	17,8	12,5	10,7	17,4	13,8	16,8	12,4	17,2	10,8	8,4	6,8	10,2	10,3	0,0	8,7	32,1	0,1	49,7	35,2	33,3
PC182 183	7,6	7,2	12,4	7,4	8,6	7,6	8,4	9,1	11,6	5,9	3,1	4,3	0,7	0,2	9,3	0,0	0,1	0,0	0,0	0,1
PC182 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PC182 204	5,7	4,7	4,5	7,2	4,9	4,0	7,3	7,9	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC182 205	9,7	8,6	3,0	5,8	4,5	3,3	4,1	5,3	2,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0	0,0	0,0
PC182 226	5,9	3,9	2,2	2,4	2,2	3,3	1,4	2,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PC183 183	0,0	1,6	5,2	0,0	2,3	0,0	2,2	0,6	5,9	1,2	0,0	0,5	0,0	0,0	3,3	0,0	0,0	0,0	0,0	0,0

PC183 205	0,0	2,4	1,9	0,0	1,7	0,0	1,6	0,7	2,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0
PC183 226	0,0	1,4	1,8	0,0	1,0	0,0	0,7	0,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0	38,4	0,1	0,8	7,0	0,0
PC204 204	0,0	0,7	0,7	1,3	0,8	0,0	1,9	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PC205 205	0,0	0,7	0,1	0,0	0,3	0,0	0,4	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,5	0,0	0,0	0,0
PC205 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PC205 226	0,0	1,0	0,3	0,0	0,4	0,0	0,3	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,9	0,0	0,0	0,0
PC226 226	0,0	0,4	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,0

Table 189: Lysophosphatidylcholin species profile of occurring LPC species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by +ESI-HPLC-MS/MS in MRM mode. Data are mean, n=2-3 and expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
LPC 14:0	0,0	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0	4,4	0,0	0,0	0,0
LPC 16:0	2,8	14,5	14,2	2,9	11,0	2,3	11,7	1,9	18,1	7,4	28,7	47,3	37,2	10,8	11,1	13,4
LPC 16:1	2,0	2,3	2,0	2,0	1,6	2,2	1,6	2,0	2,8	1,3	0,0	0,0	3,2	0,0	0,0	0,0
LPC 18:0	0,8	2,5	3,1	1,0	2,2	0,6	2,5	0,5	9,1	0,8	48,0	0,0	1,4	5,5	11,4	5,0
LPC 18:1	13,4	16,9	16,8	15,8	22,1	26,5	21,8	14,0	13,0	56,2	13,2	21,1	13,7	23,4	29,1	20,4
LPC 18:2	46,4	33,8	32,9	48,8	38,5	47,4	34,8	45,3	23,7	28,9	0,0	0,0	2,2	60,4	48,4	60,9
LPC 18:3	7,0	6,7	14,7	6,8	8,2	6,7	8,0	8,9	14,0	5,5	0,0	0,0	1,6	0,0	0,0	0,0
LPC 20:3	1,7	0,9	0,4	1,0	0,8	0,5	1,0	0,9	3,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
LPC 20:4	9,1	6,5	6,3	10,8	6,7	5,9	10,3	13,3	2,4	0,0	0,0	0,0	1,0	0,0	0,0	0,0

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LPC 20:5	5,6	6,7	3,5	4,0	3,8	2,6	3,7	4,8	5,6	0,0	10,2	21,1	22,8	0,0	0,0	0,4
LPC 22:5	1,4	0,8	0,7	1,3	0,7	0,6	1,2	3,1	0,5	0,0	0,0	0,0	1,2	0,0	0,0	0,0
LPC 22:6	9,8	8,4	5,4	5,6	4,4	4,7	3,4	5,3	7,7	0,0	0,0	10,5	11,1	0,0	0,0	0,0

Table 190: Phosphatidylethanolamine species profile of occurring PE species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by -ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil capsule	Omega 3 algae oil
PE140 160	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,0	0,0	0,0
PE140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,3	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0
PE140 182	0,0	1,1	0,6	0,4	0,6	0,0	0,5	0,4	0,5	0,4	0,0	0,4	0,5	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0
PE140 183	0,0	0,1	0,2	0,0	0,1	0,0	0,1	0,0	0,2	0,1	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,1	0,0	0,0	0,0
PE140 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0
PE140 204	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,7	0,0	0,0	0,0
PE140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	0,0	0,0	0,0
PE160 160	0,3	0,1	0,1	0,2	0,1	0,0	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,9	0,0	0,0	0,3
PE160 161	0,5	0,5	0,4	0,0	0,4	0,0	0,4	0,5	1,1	0,3	0,0	0,0	0,0	0,0	1,0	0,0	0,0	4,2	0,0	0,0	0,0
PE160 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0

PE160 181	5,0	1,9	2,4	5,4	3,0	6,6	2,6	6,0	1,8	9,7	14,7	19,0	11,4	13,0	7,2	0,0	0,0	9,9	3,1	0,0	2,5
PE160 182	26,6	22,2	17,9	24,7	17,6	23,2	21,2	22,0	26,0	9,8	13,5	12,9	12,3	4,9	19,5	0,0	0,0	6,9	25,7	35,7	30,0
PE160 183	3,0	4,6	10,4	2,9	4,7	3,7	4,8	3,7	14,6	2,4	2,4	2,0	0,3	0,0	8,4	0,0	0,0	6,1	0,0	0,0	0,0
PE160 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE160 204	1,4	2,0	1,1	1,4	1,6	0,0	2,3	1,6	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0
PE161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,1	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0
PE161 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE161 181	0,2	0,2	0,3	0,3	0,5	0,0	0,4	0,5	0,5	1,0	1,0	0,8	0,8	1,8	0,9	0,0	0,0	4,0	0,0	0,0	0,0
PE161 182	0,9	1,1	0,9	0,8	1,2	1,0	0,9	0,9	1,6	1,5	1,0	1,1	1,1	0,0	2,1	0,0	0,0	0,5	0,0	0,0	0,0
PE161 204	0,0	0,0	0,0	0,0	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0
PE180 181	0,0	0,1	0,1	0,1	0,1	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0
PE180 182	2,6	2,5	1,8	2,7	1,7	2,0	2,2	2,5	3,6	0,4	0,7	0,4	0,4	0,0	1,0	0,0	0,0	0,0	9,5	0,0	11,6
PE180 183	0,0	0,3	0,7	0,2	0,4	0,0	0,4	0,0	2,3	0,1	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0
PE180 226	0,0	0,1	0,1	0,0	0,1	0,0	0,0	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE181 181	1,9	1,0	1,2	2,4	2,8	4,6	2,0	3,4	0,6	20,6	14,1	13,2	22,3	38,9	4,9	100,0	100,0	3,4	10,0	23,0	8,7
PE181 182	13,7	12,8	13,5	14,2	16,3	17,0	14,8	14,5	6,9	26,7	27,6	25,6	35,1	28,7	19,8	0,0	0,0	1,4	10,1	2,4	7,2
PE181 183	2,2	1,9	4,8	2,6	3,3	3,2	3,7	2,9	3,8	5,2	5,6	4,0	0,8	3,9	5,2	0,0	0,0	1,2	0,0	0,0	0,0
PE181 204	0,8	0,5	0,5	1,8	1,3	1,0	1,9	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
PE181 226	0,5	0,6	0,5	0,2	0,7	0,0	0,6	0,4	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	20,9	0,0	0,0	0,0
PE182 182	17,8	21,0	11,6	16,0	18,8	19,0	15,7	15,7	13,5	14,6	13,3	15,7	13,7	7,5	16,9	0,0	0,0	0,5	41,6	38,9	39,9
PE182 183	11,0	13,1	18,2	11,5	14,1	12,3	12,0	12,0	13,7	6,3	5,6	4,6	0,9	1,4	9,4	0,0	0,0	0,0	0,0	0,0	0,0
PE182 203	0,7	0,4	0,0	0,6	0,4	0,0	0,5	0,5	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE182 204	4,7	4,9	4,4	7,1	4,0	4,7	7,0	5,7	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
PE182 225	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0
PE182 226	2,1	3,3	1,4	0,9	1,6	0,0	1,1	0,6	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	0,0	0,0	0,0
PE183 183	1,8	1,1	4,0	1,6	1,9	1,7	1,4	1,9	4,6	0,8	0,6	0,4	0,0	0,0	2,5	0,0	0,0	0,0	0,0	0,0	0,0
PE183 204	1,0	1,0	1,7	1,4	1,5	0,0	2,2	1,7	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,0

PE183 226	0,7	0,9	0,9	0,3	0,6	0,0	0,4	0,3	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,0	0,0	0,0
PE203 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0
PE203 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,0	0,0	0,0
PE204 205	0,3	0,2	0,1	0,3	0,3	0,0	0,2	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,0	0,0	0,0
PE204 226	0,0	0,3	0,2	0,1	0,2	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,1	0,0	0,0	0,0
PE205 205	0,2	0,1	0,1	0,1	0,1	0,0	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	10,6	0,0	0,0	0,0
PE226 226	0,0	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	0,0

Table 191: Lysophosphatidylethanolamine species profile of occurring LPE species comprising 12 target fatty acids from DCM/MeOH (2:1) extracts analyzed by -ESI-HPLC-MS/MS in sMRM mode. Data are mean, n=2-3 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample Name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LBFDAU oil	LBFGKN seeds	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil	Omega 3 algae oil
LPE14:0	0,0	0,2	0,2	0,0	0,1	0,0	0,1	0,0	0,2	0,1	0,1	0,1	0,0	0,1	0,0	0,6	0,0	0,0	0,0
LPE16:0	1,9	18,8	20,8	2,5	14,8	1,6	14,7	1,7	22,5	9,7	5,0	8,2	7,7	24,8	19,1	23,4	9,9	17,0	20,5
LPE16:1	0,6	0,7	0,6	0,6	0,5	0,7	0,5	0,7	0,8	1,1	0,5	0,6	0,3	1,1	0,0	1,2	0,0	0,0	0,0
LPE18:0	1,2	3,0	3,6	1,7	2,7	1,2	3,0	1,0	9,5	0,8	1,4	1,7	4,6	8,6	0,0	1,9	2,5	1,2	4,2
LPE18:1	7,1	15,1	16,4	9,8	19,2	13,6	19,5	7,6	12,1	42,1	48,5	61,1	71,7	23,6	25,6	23,7	14,8	17,9	11,9
LPE18:2	54,5	35,7	29,7	57,8	38,1	59,8	33,6	62,3	24,3	39,6	40,5	27,7	14,2	32,4	42,6	1,6	72,8	64,0	63,4
LPE18:3	6,7	6,8	13,8	7,2	9,0	7,2	8,5	8,9	12,7	6,6	4,0	0,6	1,5	9,4	0,0	0,7	0,0	0,0	0,0
LPE20:3	0,3	0,6	0,2	0,2	0,5	0,1	0,7	0,1	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0
LPE20:4	3,4	5,1	5,9	4,6	6,2	2,7	10,6	3,5	1,5	0,0	0,0	0,0	0,0	0,0	0,0	2,0	0,0	0,0	0,0

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LPE20:5	6,3	4,9	3,1	5,3	3,9	3,3	3,7	5,0	5,2	0,0	0,0	0,0	0,0	0,0	12,8	24,8	0,0	0,0	0,0
LPE22:5	0,2	0,1	0,1	0,2	0,2	0,1	0,5	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0
LPE22:6	18,0	9,0	5,5	10,1	4,8	9,6	4,3	8,4	10,2	0,0	0,0	0,0	0,0	0,0	0,0	19,3	0,0	0,0	0,0

Example 32: TAG species analysis

Diluted extracts from Example 29 were used for TAG (triacylglycerol) species analysis. TAG species analysis was carried out on a 1290 Agilent HPLC coupled to API5500 triple quadrupole MS (ABSciex). HPLC comprised of G4227A Flex Cube, G4226A autosampler, G1330B thermostat and G4220A binary pump. TAG species separation was carried out on a Thermo Accucore C30 column (250 mm x 2.1 mm x 2.6 µm). The mobile phase consisted of MeCN/IPA as solvent A and IPA as solvent B with a flow rate of 0.4 mL/min. Starting conditions were 100% followed by a linear gradient to 20% A with a total run time of 30 min. The Injection volume was 1 µL. The mass spectrometer was operated in positive APCI mode with ion source temperature of 300 °C using scheduled multiple reaction monitoring (sMRM). Data acquisition was carried out using Analyst 1.5.1 whereas data analysis was done in Multiquant 3.0.1 (AB Sciex). All theoretical $[M+H]^+$ from TAG species containing C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:3, C20:4, C20:5, C22:5 and C22:6 fatty acids were calculated resulting in a total of 364 TAG species. In MRM-mode, the neutral loss of fatty acids was monitored, therefore all theoretical product ions were calculated as well. The first quadrupole was set to filter only those $[M+H]^+$ of target TAG species that were subsequently fragmented in the second quadrupole which led to the loss of fatty acid. For TAG species containing three different fatty acids, three product ions were monitored in the third quadrupole whereas those species with two different fatty acids led to two product ions. In case of TAG species with only one fatty acid, only one transition could be monitored.

Single and mixed standards of TAGs (16:0/16:0/16:0), (16:1/16:1/16:1), (17:0/17:0/17:0), (18:0/18:0/18:0), (18:1/18:1/18:1), (18:2/18:2/18:2), (18:3/18:3/18:3), (20:5/20:5/20:5), (22:6/22:6/22:6), (16:0/16:0/18:1), (16:0/18:0/18:2), (16:0/18:1/18:1), (16:0/18:2/18:2), (18:0/18:0/18:2), (18:0/18:1/18:1), (18:0/18:1/18:2), (18:1/18:1/18:3) and (18:1/18:2/18:2) were run to identify retention time patterns among TAG species. MRMs of TAG species not present in a representative set of test samples from different canola, fish and algae oil sources were removed from the transition list. For all other species, a retention time was identified to allow for scheduled monitoring of MRM in a

given time window which allows for more data points per transition thus leading to a better quality of data.

Baseline separation of all TAG species could not achieved within a reasonable runtime, therefore peak heights were used instead of peak areas. The sums of all transitions with were used to calculate a total peak height. Species with peak height lower than 1000 counts were not included in the calculation as they were considered below limit of quantitation having a poor signal-to-noise ratio. TAG species profile was then calculated as TAG species percent of total TAG species.

The results are shown in Table 192.

Table 192: Triacylglycerol sspecies profile of occurring TAG species comprising 12 target fatty acids from MTBE extracts analyzed by +APCI-HPLC-MS/MS in sMRM mode. Data are mean, n=2 expressed as relative abundance of species. Species that were not detected in any samples were removed from the table

Sample name	LANBCH oil	LANPMZ seeds	LAODDN seeds	LANBCH oil	LBFGKN oil	LBFLFK seeds	LBFLFK oil	Arabidopsis seeds	Kumily seeds	Kumily oil	Canola Commodity oil	Clear Valley 65 oil	Clear Valley 80 oil	Brassica juncea seeds	Menhaden oil	Salmon oil capsule	Krill oil capsule	DHA DHA oil capsule	Omega EPA/DHA oil	Omega 3 algae oil
TAG 140 140 140	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,1
TAG 140 140 160	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	0,1
TAG 140 140 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,8	0,7	10,8	0,0	0,0	0,0
TAG 140 140 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,4	10,5	0,0	0,0	0,0
TAG 140 140 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,2	2,1	0,0	0,0	0,0
TAG 140 140 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,7	7,0	3,3	0,3	0,4	0,4
TAG 140 140 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,5	0,0	2,7
TAG 140 140 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	2,0	1,2	10,5	1,0	10,4
TAG 140 160 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,6	15,4	0,0	0,0	0,0
TAG 140 160 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	1,4	15,7	0,0	0,0	0,0
TAG 140 160 182	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	2,5	0,0	0,0	0,0
TAG 140 160 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,3	10,1	8,8	0,0	2,0	0,0
TAG 140 160 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,1	4,4	2,5	23,9	3,7	21,2
TAG 140 161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,5	0,7	2,4	0,0	0,0	0,0
TAG 140 161 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,3	0,0	0,0	0,0	0,0
TAG 140 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	8,4	7,0	2,9	0,0	0,0	0,0
TAG 140 161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,7	1,7	0,0	0,0	0,0	0,4
TAG 140 181 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,4	5,0	0,0	0,0	0,0

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TAG 140 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,6	3,4	0,0	0,1	1,2	0,2
TAG 140 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	2,2	0,0	1,3	3,6	1,1
TAG 160 160 181	0,1	0,1	0,2	0,1	0,1	0,1	0,0	0,1	0,2	0,2	0,1	0,3	0,2	0,5	0,2	0,3	0,9	0,0	0,0	0,0
TAG 160 160 182	0,5	0,3	0,3	0,5	0,4	0,3	0,4	1,1	0,4	0,2	0,4	0,3	0,2	2,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 160 183	0,3	0,2	0,2	0,3	0,3	0,3	0,3	2,4	0,8	0,5	0,5	0,0	0,0	7,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 160 205	0,2	0,2	0,2	0,2	0,1	0,3	0,2	0,6	0,0	0,0	0,0	0,0	0,0	0,0	2,9	3,6	1,4	0,0	5,9	0,0
TAG 160 160 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	0,0
TAG 160 160 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	2,6	0,0	11,3	12,6	8,8
TAG 160 161 161	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,8	0,8	0,0	0,0	0,0	0,0
TAG 160 161 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,4	0,7	3,3	0,0	0,0	0,0
TAG 160 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	6,1	8,3	1,5	0,0	0,0	0,0
TAG 160 161 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,7	1,0	0,0	0,0	0,0	0,0
TAG 160 161 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	5,7	0,0	0,0	1,1	0,3	0,7
TAG 160 180 181	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,3	0,2	0,2	0,2	0,3	0,5	0,4	0,1	0,1	0,1	0,1	0,1	0,1
TAG 160 180 182	0,1	0,2	0,3	0,1	0,1	0,1	0,1	0,5	0,1	0,1	0,1	0,1	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 180 183	0,2	0,2	0,2	0,2	0,2	0,2	0,1	2,6	0,3	0,2	0,2	0,0	0,1	1,9	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 180 205	0,0	0,1	0,1	0,0	0,0	0,1	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,5	0,0	0,0	0,8	0,0
TAG 160 180 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0
TAG 160 180 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	0,0
TAG 160 181 181	0,7	2,4	0,8	0,7	0,9	0,8	1,0	0,5	3,4	5,1	2,5	4,7	12,5	1,7	0,2	0,4	1,3	0,8	2,0	1,1
TAG 160 181 182	2,6	1,9	3,4	2,6	2,7	3,2	2,3	2,0	4,8	2,8	3,7	8,4	3,0	4,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 181 183	1,3	1,8	0,0	1,3	1,8	1,5	0,0	3,1	6,6	5,1	6,1	1,0	3,4	9,8	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 181 205	1,5	1,3	1,2	1,5	1,2	2,0	2,2	1,3	0,0	0,0	0,0	0,0	0,0	0,0	4,9	8,1	3,0	0,0	0,0	0,0
TAG 160 181 225	0,7	0,4	0,7	0,7	0,4	0,7	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 182 182	4,1	4,9	4,3	4,1	2,9	3,2	2,1	4,1	1,8	1,6	0,0	1,9	0,0	5,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 182 183	2,4	2,5	3,1	2,4	2,6	2,3	1,5	9,2	5,8	3,7	3,5	0,5	0,4	15,4	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 182 205	4,3	3,3	3,1	4,3	1,7	3,8	2,4	7,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 182 225	1,7	1,6	2,1	1,7	0,8	1,4	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

TAG 160 183 183	0,3	0,3	0,5	0,3	0,6	0,4	0,4	4,6	1,1	1,2	0,0	0,0	0,0	10,2	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 183 205	0,7	0,4	0,5	0,7	0,6	0,6	0,7	2,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 160 184 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,7	2,9	2,0	0,0	0,0	0,0
TAG 160 205 205	0,4	0,2	0,2	0,4	0,3	0,4	0,3	1,6	0,0	0,0	0,0	0,0	0,0	0,0	2,8	3,6	0,9	0,0	8,0	0,0
TAG 160 205 225	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,1	0,0
TAG 160 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0	2,4	3,8	0,0	0,0	13,4	0,0
TAG 160 225 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	13,0	1,2	16,5
TAG 160 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,0	1,1	0,0	21,2	12,0	20,1
TAG 161 161 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,3	0,0	0,0	0,0	0,0
TAG 161 161 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	4,9	3,4	0,0	0,0	0,0	0,0
TAG 161 181 181	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,2	0,1	0,7	0,6	0,0	0,1	0,2	0,7	0,0	0,0	0,0
TAG 161 181 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 181 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,0	3,1	0,0	0,0	0,0	0,0
TAG 161 182 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 161 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,6	1,7	0,0	0,0	0,0	0,0
TAG 161 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,1	0,0	0,0	0,0	0,0	0,0
TAG 161 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,3	0,0	0,6	0,1	0,5
TAG 180 180 180	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 180 181	0,1	0,1	0,1	0,1	0,2	0,1	0,1	0,4	0,2	0,2	0,1	0,3	0,7	0,2	0,0	0,0	0,0	0,1	0,1	0,1
TAG 180 180 182	0,1	0,1	0,1	0,1	0,1	0,1	0,1	0,5	0,0	0,0	0,1	0,0	0,1	0,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 180 183	0,0	0,0	0,0	0,0	0,1	0,0	0,0	0,6	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 181	0,2	0,3	0,2	0,2	0,3	0,2	0,2	0,2	0,8	0,6	0,3	1,4	2,8	0,0	0,0	0,0	0,0	0,4	0,6	0,4
TAG 180 181 182	0,5	0,8	0,4	0,5	0,6	0,5	0,4	0,8	0,7	0,9	0,9	1,3	1,7	0,0	0,0	0,0	0,0	0,0	0,2	0,0
TAG 180 181 183	0,9	0,4	0,4	0,9	0,8	0,4	0,4	1,4	2,0	0,9	0,9	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 181 205	0,8	0,4	0,4	0,8	0,4	0,9	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0
TAG 180 181 225	0,2	0,1	0,1	0,2	0,1	0,1	0,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,2	0,0	0,0	0,0	0,0
TAG 180 182 182	0,9	0,8	1,0	0,9	0,9	0,8	0,6	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0

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TAG 180 182 183	0,8	0,6	0,8	0,8	0,7	0,7	0,6	5,0	0,6	0,5	0,6	0,0	0,0	2,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 182 205	0,7	0,4	0,4	0,7	0,6	0,7	0,7	1,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 182 225	0,6	0,3	0,5	0,6	0,3	0,9	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 183 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,6	0,3	0,3	0,0	0,0	0,0	1,4	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 183 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 180 205 205	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,7	0,0	0,0	0,0	0,0
TAG 180 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,9	0,0	0,0	0,7	0,0
TAG 180 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,2	0,0	0,5	0,4	0,4
TAG 181 181 181	1,2	1,4	1,1	1,2	2,9	1,3	2,6	0,5	12,4	8,2	0,0	20,9	29,9	0,3	0,1	0,2	0,3	3,3	5,0	3,3
TAG 181 181 182	2,1	3,8	4,6	2,1	3,7	3,5	2,6	0,5	10,8	9,1	17,1	33,1	18,8	1,7	0,0	0,0	0,0	0,7	1,1	0,4
TAG 181 181 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	23,1	18,4	6,2	13,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 181 203	0,6	0,0	0,5	0,6	0,7	1,0	1,1	0,0	0,0	0,0	4,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 181 204	1,5	0,0	0,0	1,5	1,7	0,0	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 181 205	5,3	6,6	7,5	5,3	6,9	4,9	7,6	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,4	1,3	0,0	0,0	0,0	0,0
TAG 181 181 225	1,9	2,5	1,5	1,9	1,0	1,4	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,1	0,0	0,0	0,0	0,0
TAG 181 181 226	0,5	0,7	0,3	0,5	1,0	0,8	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,8	0,0	0,0	0,0	0,0
TAG 181 182 182	5,2	8,0	9,9	5,2	8,1	6,1	4,9	0,0	13,5	5,5	5,0	13,1	4,9	0,0	0,0	0,0	0,0	0,5	0,6	0,3
TAG 181 182 183	5,7	5,8	4,6	5,7	6,5	5,8	6,0	3,1	16,4	18,6	13,3	3,6	3,8	8,7	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 182 203	0,9	1,6	1,6	0,9	1,3	2,0	1,6	0,0	0,0	0,0	1,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 182 205	10,1	10,2	10,5	10,1	7,5	12,4	10,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 182 225	4,2	3,0	3,8	4,2	2,7	3,5	3,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 182 226	1,4	0,9	0,8	1,4	1,2	2,1	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 183 183	0,0	0,0	0,0	0,0	0,0	0,0	0,0	2,9	8,0	4,1	4,5	0,0	1,3	4,4	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	3,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 204 205	1,2	0,0	0,0	1,2	1,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 181 205 205	1,1	0,8	1,0	1,1	1,2	1,8	1,3	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,8	1,2	0,3	0,0	0,0	0,0
TAG 181 205 225	0,6	0,4	0,4	0,6	0,5	0,8	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,3	0,0	0,0	0,0	0,0
TAG 181 205 226	0,4	0,3	0,1	0,4	0,1	0,2	0,2	0,6	0,0	0,0	0,0	0,0	0,0	0,0	0,9	1,6	0,0	0,0	0,0	0,0

TAG 181 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,3	0,0	0,0	0,0	0,0
TAG 182 182 182	4,2	6,4	5,5	4,2	3,0	3,5	6,0	0,8	2,4	1,7	0,0	1,6	0,3	2,0	0,0	0,0	0,0	0,3	0,5	0,6
TAG 182 182 183	3,3	3,5	5,7	3,3	5,3	3,4	3,9	1,9	2,9	2,7	3,4	0,3	0,4	8,4	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 182 203	1,0	0,0	0,0	1,0	0,8	0,0	1,9	0,0	0,0	0,0	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 182 205	5,3	8,1	3,6	5,3	7,3	5,1	6,9	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 182 225	2,6	2,1	2,5	2,6	2,1	2,6	1,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 182 226	1,2	1,1	0,6	1,2	1,5	1,2	1,4	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 183 183	1,5	1,0	1,5	1,5	2,3	1,4	1,1	2,8	2,0	2,1	2,0	0,0	0,2	7,5	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	1,6	0,0	0,0	2,4	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 183 205	2,7	2,0	2,8	2,7	2,7	3,4	3,5	4,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 183 225	1,6	1,0	1,3	1,6	0,7	0,0	0,9	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 183 226	0,9	0,0	0,0	0,9	1,1	0,0	0,8	2,7	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 205 205	0,9	0,5	0,6	0,9	0,7	1,1	1,7	2,1	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 205 225	1,1	0,5	0,4	1,1	0,5	1,1	1,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 205 226	0,0	0,3	0,2	0,0	0,0	0,4	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 182 225 226	0,2	0,1	0,1	0,2	0,1	0,2	0,2	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 183 183 183	0,1	0,0	0,2	0,1	0,3	0,2	0,1	0,7	0,6	0,5	0,0	0,0	0,0	1,3	0,0	0,0	0,0	0,0	0,0	0,0
TAG 183 183 203	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 183 183 205	0,4	0,2	0,4	0,4	0,3	0,5	0,4	2,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 183 183 226	0,1	0,1	0,1	0,1	0,1	0,1	0,1	1,3	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 183 205 205	0,4	0,1	0,2	0,4	0,3	0,5	0,3	1,5	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,4	0,0	0,0	0,0	0,0
TAG 183 205 226	0,0	0,1	0,1	0,0	0,0	0,2	0,0	0,8	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0
TAG 205 205 205	0,2	0,1	0,1	0,2	0,1	0,2	0,1	0,7	0,0	0,0	0,0	0,0	0,0	0,0	0,6	0,9	0,4	0,0	2,5	0,0
TAG 205 205 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,3	0,0	0,0	0,0	0,0	0,0	0,0	0,5	0,8	0,2	0,5	5,1	0,8
TAG 205 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,2	0,5	0,0	1,0	5,3	1,5
TAG 226 226 226	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,0	0,1	0,0	4,7	3,8	7,7

CSIRO Exhibit 1004

Sequences of the invention

Table 193 shows the sequences of the invention by their name, and the associated SEQ-ID number of the sequence lisiting.

Table 193: Assosiation of sequence names to SEQ-IDs in the sequence listing.

			Continued from column 1 and 2	
SEQ ID NO:	**Sequence Name**		**SEQ ID NO:**	**Sequence Name**
1	RTP10690-1qcz_F		129	j-i-Atss1_c-d5Elo(Ot_GA3)_P
2	RTP10691-2qcz		130	j-i-Atss1_c-d6Elo(Tp_GA2)_P
3	VC-LTM593-1qcz		131	j-i-Atss14_c-d12Des(Ps_GA)_P
4	VC-LTM595-1qcz		132	j-i-Atss18_c-d6Elo(Pp_GA2)_P
5	VC-LJB2197-1qcz		133	j-i-Atss18_c-o3Des(Pi_GA2)_P
6	VC-LJB2755-2qcz		134	j-i-Atss2_c-d4Des(Tc_GA3)_P
7	VC-LLM306-1qcz		135	j-i-Atss2_c-d6Des(Ot febit_GA)_P
8	VC-LLM337-1qcz		136	j-p-BnFAE_ t-PvARC_P
9	VC-LLM338-3qcz		137	j-p-BnSETL-v1_c-o3Des(Pir_GA)_P
10	VC-LLM391-2qcz		138	j-p-LuPXR_i-Atss15_P
11	VC-LLM217-1qcz		139	j-p-PvARC5_t-BnFAE_P
12	o3DES-S94FAM		140	j-p-PvARC5_t-BnSETL_P
13	o3DESPIR-198FAM		141	j-t-CaMV_p-LuCnl-2_P
14	o3DESPIR-962FAM		142	j-t-E9-p3-2_P
15	AtPXR-Fam		143	j-t-PvARC-p-LuCnl_P
16	BnSETL-1186FAM		144	j-t-StCAT_p2_p-LuPXR_P
17	BnSETL-Fam		145	reference (Adh1)_P
18	E011		146	c-o3Des(Pi_GA2_SNP)_F
19	D4DES-Tc-FAM		147	j-t-PvARC-p3_F
20	D4DFS-Eg-FAM		148	j-i-Atss15_c-o3Des(Pi_GA2)_F
21	D4DES-PI-770FAM		149	j-p-VfSBPperm3_c-o3Des(Pir_GA)_F
22	Exp3-78-YAK		150	c-d15Des(Ch_ERTp_GA)_F
23	o3DES-572F		151	j-i-Atss2_c-d4Des(PI_GA)-195R)_F
24	o3DESPIR-160F		152	c-o3Des(Pi_GA2_SNP)_R
25	o3DESPIR-924F		153	j-t-PvARC-p3_R
26	o3DesPir-F		154	j-i-Atss15_c-o3Des(Pi_GA2)_R
27	BnSETL-1164-F		155	j-p-VfSBPperm3_c-o3Des(Pir_GA)_R
28	MA54		156	c-d15Des(Ch_ERTp_GA)_R
29	D4DES-Tc-F		157	j-i-Atss2_c-d4Des(PI_GA)-195R)_R
30	D4DES-EG-F		158	c-o3Des(Pi_GA2_SNP)_P
31	D4DES-PI-746-F		159	j-t-PvARC-p3_P
32	Exp 3-52F		160	j-i-Atss15_c-o3Des(Pi_GA2)_P
33	o3DES-652R		161	j-p-VfSBPperm3_c-o3Des(Pir_GA)_P
34	o3DESPIR-262R		162	c-d15Des(Ch_ERTp_GA)_P
35	o3DESPIR-1026-R		163	j-i-Atss2_c-d4Des(PI_GA)-195R)_P
36	AtPXR-R		164	LANBCH locus 1 (N5) Forward primer
37	o3DESPIR-R		165	LANBCH locus 1 (N9) Forward primer
38	MA55		166	LANBCH locus 2 (N5) Forward primer
39	D4DES-Tc-988R		167	LANBCH locus 2 (N9) Forward primer

SEQ ID NO:	Sequence Name		SEQ ID NO:	Sequence Name
			Continued from column 1 and 2	
40	D4DES-Eg-R		168	LBFDAU locus 1 Forward primer
41	D4DES-PI-817R		169	LBFDAU locus 2 Forward primer
42	Exp 3-128R		170	LBFDGG Forward primer
43	RTP10690_1		171	LBFDHG locus 1 Forward primer
44	RTP10690_2		172	LBFDHG locus 2 Forward primer
45	RTP10690_3		173	LBFGKN Forward primer
46	RTP10690_4		174	LBFIHE Forward primer
47	RTP10690_5		175	LBFLFK locus 1 Forward primer
48	RTP10690_6		176	LBFLFK locus 2 Forward primer
49	RTP10690_7		177	LBFPRA Locus 1 Forward primer
50	RTP10690_8		178	LBFPRA locus 2 Forward primer
51	RTP10690_9		179	LBFPRA Locus 3e Forward primer
52	RTP10690_10		180	LANBCH locus 1 (N5) Reverse primer
53	RTP10690_11		181	LANBCH locus 1 (N9) Reverse primer
54	RTP10690_12		182	LANBCH locus 2 (N5) Reverse primer
55	RTP10690_5'		183	LANBCH locus 2 (N9) Reverse primer
56	t-AtAHASL		184	LBFDAU locus 1 Reverse primer
57	LJB2197_5'		185	LBFDAU locus 2 Reverse primer
58	LJB2197_1		186	LBFDGG Reverse primer
59	LJB2197_2		187	LBFDHG locus 1 Reverse primer
60	LJB2197_3		188	LBFDHG locus 2 Reverse primer
61	LJB2197_4		189	LBFGKN Reverse primer
62	c-AHAS_F		190	LBFIHE Reverse primer
63	c-d4Des(Eg_GA)_F		191	LBFLFK locus 1 Reverse primer
64	c-d4Des(PI_GA)2_F		192	LBFLFK locus 2 Reverse primer
65	c-d4Des(Tc_GA)_F		193	LBFPRA Locus 1 Reverse primer
66	c-d5Des(Tc_GA)_F		194	LBFPRA locus 2 Reverse primer
67	c-d5Elo(Ot_GA3)_F		195	LBFPRA Locus 3e Reverse primer
68	c-d6Des(Ot_febit)_F		196	LANBCH locus 1 (N5) Probe sequence
69	c-d6Elo(Pp_GA)_F		197	LANBCH locus 1 (N9) Probe sequence
70	c-d6Elo(Tp_GA)_F		198	LANBCH locus 2 (N5) Probe sequence
71	c-o3Des(Pi_GA)_F		199	LANBCH locus 2 (N9) Probe sequence
72	c-o3Des(Pir_GA)_F		200	LBFDAU locus 1 Probe sequence
73	j-i-Atss1_c-d5Elo(Ot_GA3)_F		201	LBFDAU locus 2 Probe sequence
74	j-i-Atss1_c-d6Elo(Tp_GA2)_F		202	LBFDGG Probe sequence
75	j-i-Atss14_c-d12Des(Ps_GA)_F		203	LBFDHG locus 1 Probe sequence
76	j-i-Atss18_c-d6Elo(Pp_GA2)_F		204	LBFDHG locus 2 Probe sequence
77	j-i-Atss18_c-o3Des(Pi_GA2)_F		205	LBFGKN Probe sequence
78	j-i-Atss2_c-d4Des(Tc_GA3)_F		206	LBFIHE Probe sequence
79	j-i-Atss2_c-d6Des(Ot febit_GA)_F		207	LBFLFK locus 1 Probe sequence
80	j-p-BnFAE_t-PvARC_F		208	LBFLFK locus 2 Probe sequence
81	j-p-BnSETL-v1_c-o3Des(Pir_GA)_F		209	LBFPRA Locus 1 Probe sequence
82	j-p-LuPXR_i-Atss15_F		210	LBFPRA locus 2 Probe sequence
83	j-p-PvARC5_t-BnFAE_F		211	LBFPRA Locus 3e Probe sequence
84	j-p-PvARC5_t-BnSETL_F		212	VC-LJB2197-1qcz LANBCH RB6 Locus 1
85	j-t-CaMV_p-LuCnl-2_F		213	VC-LJB2197-1qcz LANBCH LB2 Locus 1

			Continued from column 1 and 2	
SEQ ID NO:	**Sequence Name**		**SEQ ID NO:**	**Sequence Name**
86	j-t-E9-p3-2_F		214	VC-LLM337-1qcz LANBCH RB2 Locus 1
87	j-t-PvARC-p-LuCnl_F		215	VC-LLM337-1qcz LANBCH LB1 Locus 1
88	j-t-StCAT_p2_p-LuPXR_F		216	VC-LJB2197-1qcz LANBCH RB3 Locus 2
89	reference (Adh1)_F		217	VC-LLM337-1qcz LANBCH RB1 Locus 2
90	c-AHAS_R		218	VC-LTM593-1qcz LBFDGG RB
91	c-d4Des(Eg_GA)_R		219	VC-LTM593-1qcz LBFDGG LB
92	c-d4Des(Pl_GA)2_R		220	VC-LTM593-1qcz LBFDHG RB Locus 1
93	c-d4Des(Tc_GA)_R		221	VC-LTM593-1qcz LBFDHG LB Locus 1
94	c-d5Des(Tc_GA)_R		222	VC-LTM593-1qcz LBFDHG RB Locus 2
95	c-d5Elo(Ot_GA3)_R		223	VC-LTM593-1qcz LBFDHG LB Locus 2
96	c-d6Des(Ot_febit)_R		224	VC-LTM593-1qcz LBFGKN RB1
97	c-d6Elo(Pp_GA)_R		225	VC-LTM593-1qcz LBFGKN LB1
98	c-d6Elo(Tp_GA)_R		226	VC-LTM593-1qcz LBFIHE RB1
99	c-o3Des(Pi_GA)_R		227	VC-LTM593-1qcz LBFIHE LB1
100	c-o3Des(Pir_GA)_R		228	VC-LTM593-1qcz LBFLFK RB1 Locus 1
101	j-i-Atss1_c-d5Elo(Ot_GA3)_R		229	VC-LTM593-1qcz LBFLFK LB1 Locus 1
102	j-i-Atss1_c-d6Elo(Tp_GA2)_R		230	VC-LTM593-1qcz LBFLFK RB2 Locus 2
103	j-i-Atss14_c-d12Des(Ps_GA)_R		231	VC-LTM593-1qcz LBFLFK LB2 Locus 2
104	j-i-Atss18_c-d6Elo(Pp_GA2)_R		232	VC-LTM593-1qcz LBFPRA RB1 Locus 1
105	j-i-Atss18_c-o3Des(Pi_GA2)_R		233	VC-LTM593-1qcz LBFPRA LB1 Locus 1
106	j-i-Atss2_c-d4Des(Tc_GA3)_R		234	VC-LTM593-1qcz LBFPRA RB3 Locus 2
107	j-i-Atss2_c-d6Des(Ot febit_GA)_R		235	VC-LTM593-1qcz LBFPRA L1 Locus 2
108	j-p-BnFAE_t-PvARC_R		236	VC-LTM593-1qcz LBFPRA Locus 3e
109	j-p-BnSETL-v1_c-o3Des(Pir_GA)_R		237	VC-LTM593-1qcz LBFDAU RB1 Locus 1
110	j-p-LuPXR_i-Atss15_R		238	VC-LTM593-1qcz LBFDAU LB1 Locus 1
111	j-p-PvARC5_t-BnFAE_R		239	VC-LTM593-1qcz LBFDAU R Locus 2
112	j-p-PvARC5_t-BnSETL_R		240	VC-LTM593-1qcz LBFDAU L Locus 2
113	j-t-CaMV_p-LuCnl-2_R		241	Amplicon LANBCH locus 1 (N5)
114	j-t-E9-p3-2_R		242	Amplicon LANBCH locus 1 (N9)
115	j-t-PvARC-p-LuCnl_R		243	Amplicon LANBCH locus 2 (N5)
116	j-t-StCAT_p2_p-LuPXR_R		244	Amplicon LANBCH locus 2 (N9)
117	reference (Adh1)_R		245	Amplicon LBFDAU locus 1
118	c-AHAS_P		246	Amplicon LBFDAU locus 2
119	c-d4Des(Eg_GA)_P		247	Amplicon LBFDGG
120	c-d4Des(Pl_GA)2_P		248	Amplicon LBFDHG locus 1
121	c-d4Des(Tc_GA)_P		249	Amplicon LBFDHG locus 2
122	c-d5Des(Tc_GA)_P		250	Amplicon LBFGKN
123	c-d5Elo(Ot_GA3)_P		251	Amplicon LBFIHE
124	c-d6Des(Ot_febit)_P		252	Amplicon LBFLFK locus 1
125	c-d6Elo(Pp_GA)_P		253	Amplicon LBFLFK locus 2
126	c-d6Elo(Tp_GA)_P		254	Amplicon LBFPRA Locus 1
127	c-o3Des(Pi_GA)_P		255	Amplicon LBFPRA locus 2
128	c-o3Des(Pir_GA)_P		256	Amplicon LBFPRA Locus 3e

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Claims

1. T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and at lest one expression cassette comprising a promotor, operatively linked thereto a target gene, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp, and the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

2. T-DNA comprising the coding sequences of any single of the tables given in the examples, preferably comprising the coding sequences and and promotors of any single of the tables given in the examples, more preferably the coding sequences and promotors and terminators of any single of the tables given in the examples, and most preferably the expression cassettes of any single of the tables of the examples.

3. Plant or a seed or part thereof, comprising, integrated in its genome, a heterologous T-DNA of the present invention.

4. Plant comprising one or more T-DNA comprising one or more expression cassetts encoding for one or more d5Des, one or more d6Elo, one or more d5Des, one or more o3Des, one or more d5Elo and one or more D4Des.

5. The plant of claim 3 or 4 comprising one or more T-DNAs encoding for at least two d6Des, at least two d6Elo and/or, at least two o3Des.

6. The plant any one of claims 3 to 5 on or a part thereof comprising a T-DNA encoding for at least one CoA-depenent d4Des and at least one phopho-lipid dependent d4Des.

7 The plant, seed or part thereof any one of claims 3 to 6 the plant, seed or part thereof further encoding for one or more d12Des.

8. Plant or seed thereof of family Brassicaceae, preferably of genus Brassica, with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

   i) crossing a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant comprising a T-DNA of any of claims 1 or 2 and/or part of such T-DNA, with a plant of family Brassicaceae, preferably of genus Brassica, most preferably of genus **Brassica** napus, Brassica oleracea, Brassica nigra or Brassica carinata, said plant not comprising said T-DNA and/or part thereof, to yield a F1 hybrid,

   ii) selfing the F1 hybrid for at least one generation, and

   iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of **EPA is at least 6%, preferably at least 7,5%** (w/w) and/or the content of DHA is at least **0,8% (w/w), preferably 1,2%** of the total seed fatty acid content at an oil content of 40% (w/w).

9. Method for creating a plant with a genotype that confers a heritable phenotype of seed oil VLC-PUFA content, obtainable or obtained from progeny lines prepared by a method comprising the steps of

   i) crossing a transgenic plant according to any of claims 3 or 4 with a plant not comprising a T-DNA according to any of claims 1 or 2 or part thereof, said latter plant being of family Brassicaceae, preferably of genus Brassica, most preferably of genus Brassica napus, Brassica oleracea, Brassica nigra or Brassica carinata, to yield a F1 hybrid,

   ii) selfing the F1 hybrid for at least one generation, and

   iii) identifying the progeny of step (ii) comprising the T-DNA of the present invention capable of producing seed comprising VLC-PUFA such that the content of all VLC-PUFA downstream of 18:1n-9 is at least 40% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w), or preferably the content of **EPA is at least 6%, preferably at least 7,5% (w/w) and/or the**

content of DHA is at least 0,8% (w/w), prerferably 1.2% (w/w) of the total seed fatty acid content at an oil content of 40% (w/w).

10. The method of claim 9 o the plant of claim 8, wherein the T-DNA is homozygous

11. Method of plant oil production, comprising the steps of
    i) growing a plant according to any of claims 3 to 8 such as to obtain oil-containing seeds thereof,
    ii) harvesting said seeds, and
    iii) extracting oil from said seeds harvested in step ii), wherein the oil has a DHA content of at least 1% by weight based on the total lipid content and/or a EPA content of at least 8% by weight based on the total lipid content.

12. Plant oil comprising a polyunsaturated fatty acid obtainable or obtained by the method of claim 11.

13. Method for analysing desaturase reaction specificity, comprising the steps of
    i) providing, to a desaturase, a detectably labelled molecule comprising a fatty acid moiety and a headgroup,
    ii) allowing the desaturase to react on the labelled molecule, and
    iii) detecting desaturation products.

14. Method for analysing elongase reaction specificity, comprising the steps of
    i) providing, to an elongase, a detectably labelled elongation substrate and a molecule to be elongated,
    ii) allowing the elongase to elongate the molecule to be elongated using the labelled elongation substrate, and
    iii) detecting elongation products.

15. Method for optimization of a metabolic pathway, comprising the steps of
    i) providing enzymes of a metabolic pathway and one or more substrates to be used by the first enzyme or enzymes of the pathway,
    ii) reacting the enzymes and the substrates to produce products, which in turn are also exposed as potential substrates to the enzymes of the pathway, and

iii) determining the accumulation of products.

16. Method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to produce a substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

ii) providing a non-CoA dependent desaturase to produce the substrate for the target desaturase, and determining conversion efficiency of the target desaturase, and

iii) comparing the target desaturase conversion efficiencies of step i) and ii).

17. Method for determining CoA-dependence of a target desaturase, comprising the steps of

i) providing an elongase to elongate the products of the target desaturase, and determining conversion efficiency of the elongase,

ii) providing the elongase to elongate the products of a comparison desaturase known to be non-CoA dependent, and determining conversion efficiency of the elongase,

iii) comparing the elongase conversion efficiencies of step i) and ii).

Abstract

MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

T-DNA for expression of a target gene in a plant, wherein the T-DNA comprises a left and a right border element and an expression cassette comprising a promotor, operatively linked thereto a target gene or a combination of target genes, and downstream thereof a terminator, wherein the length of the T-DNA, measured from left to right border element and comprising the target gene, has a length of at least 30000 bp, and the expression cassette is separated from the closest border of the T-DNA by a separator of at least 500 bp length.

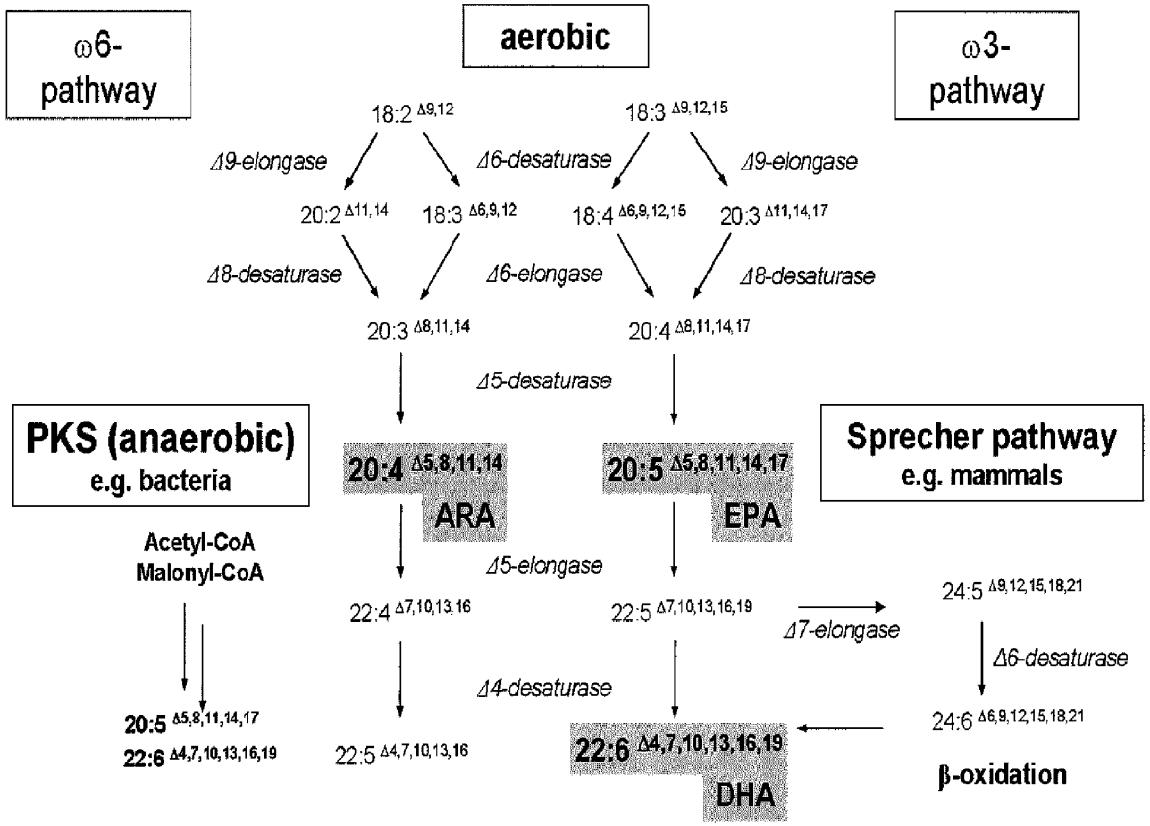

Figure 1: Schematical figure of the different enzymatic activities leading to the production of ARA, EPA and DHA

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Formula used for pathway step conversion efficiency Ceff		$C_{eff} = \frac{100}{S + P} \times P$						
Pathway Step	**Product Stream**	**pre-requisite of product stream**						
d12Des	d12Des Product Stream 1	18:1n-9	18:1n-9	18:2n-6	18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6
	d12Des Product Stream 2	18:3n-3 from 18:2n-6 via d15Des		18:3n-3	18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3
	d12Des Product Stream 3	20:2n-6 from 18:2n-5 via d6Elo		20:2n-6					
	d12Des Product Stream 4	20:3n-3 from 18:3n-3 via d6Elo		20:3n-3					
d6Des	d12Des Product Stream 1	18:2n-6		18:2n-6	18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6
	d12Des Product Stream 2	18:3n-3 produced by o3Des		18:3n-3	18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3
d6Elo	d6Elo Product Stream 1	18:3n-6 produced by d6Des			18:3n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6
	d6Elo Product Stream 2	18:4n-3 produced by d6Des or o3Des			18:4n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3
d5Des	d5Des Product Stream 1	20:3n-6 produced by d6Elo				20:3n-6	20:4n-6	22:4n-6	22:5n-6
	d5Des Product Stream 2	20:4n-3 produced by d6Elo or o3Des				20:4n-3	20:5n-3	22:5n-3	22:6n-3
d5Elo	d5Elo Product Stream 1	20:4n-6 produced by d5Des					20:4n-6	22:4n-6	22:5n-6
	d5Elo Product Stream 2	20:4n-3 produced by d5Des or o3Des					20:5n-3	22:5n-3	22:6n-3
d4Des	d4Des Product Stream 1	20:4n-6 produced by d5Des						22:4n-6	22:5n-6
	d4Des Product Stream 2	20:4n-3 produced by d5Des or o3Des						22:5n-3	22:6n-3
o3Des		List of all o6Des Fatty acid substrates	18:2n-6	18:3n-6	20:2n-6	20:3n-6	20:4n-6	22:4n-6	22:5n-6
		List of all o3Des Fatty acid products	18:3n-3	18:4n-3	20:3n-3	20:4n-3	20:5n-3	22:5n-3	22:6n-3

Figure 2: Formulas to calculate pathway step conversion efficiencies. S: substrate of pathway step. P: product of pathway step. Product was always the sum of the immediate product of the conversion at this pathway step, and all downstream products that passed this pathway step in order to be formed. E.g. DHA (22:6n-3 does possess a double bond that was a result of the delta-12-desaturation of oleic acid (18:1n-9) to linoleic acid (18:2n-6).

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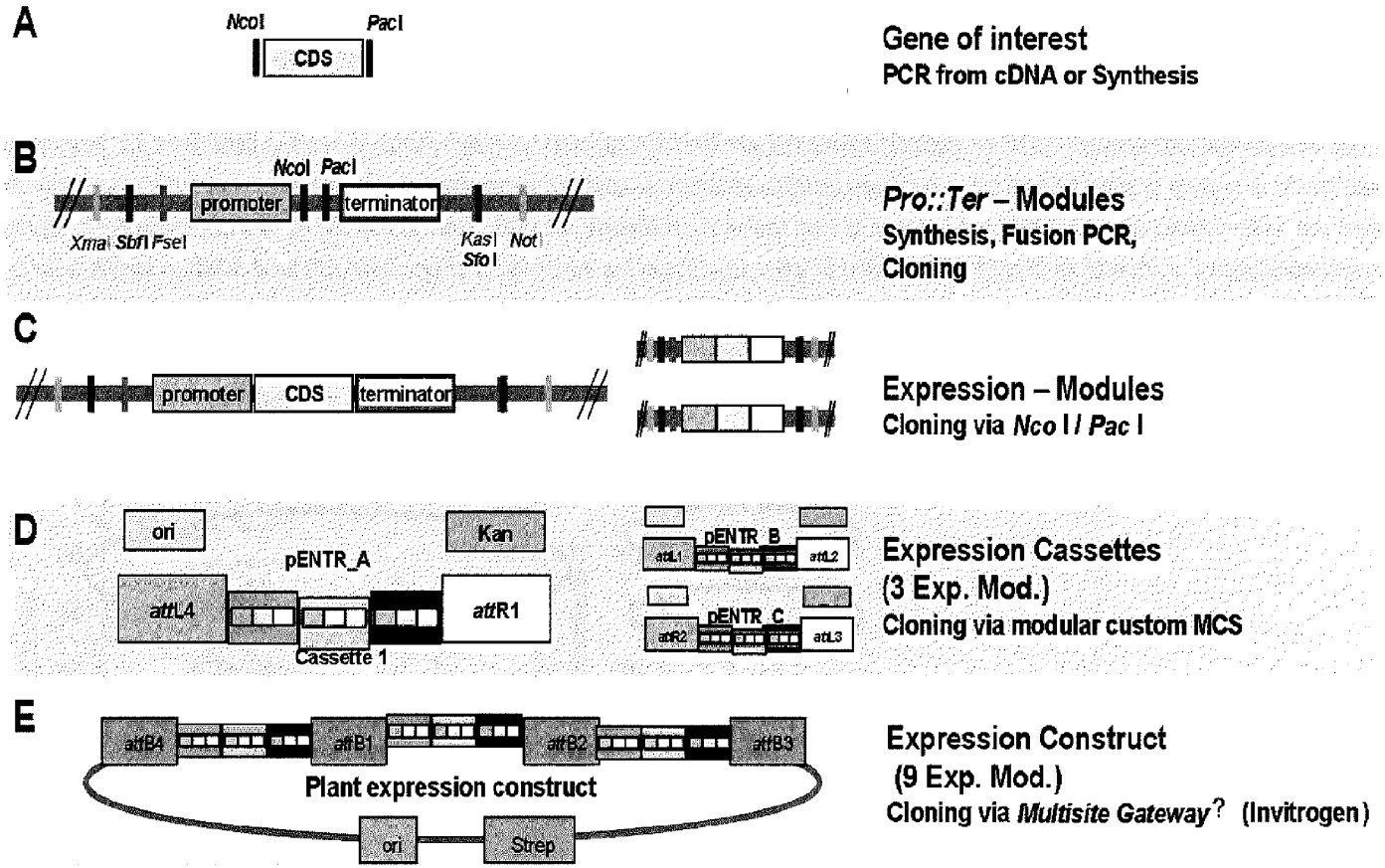


Figure 3: Strategy employed for stepwise buildup of plant expression plasmids of the invention

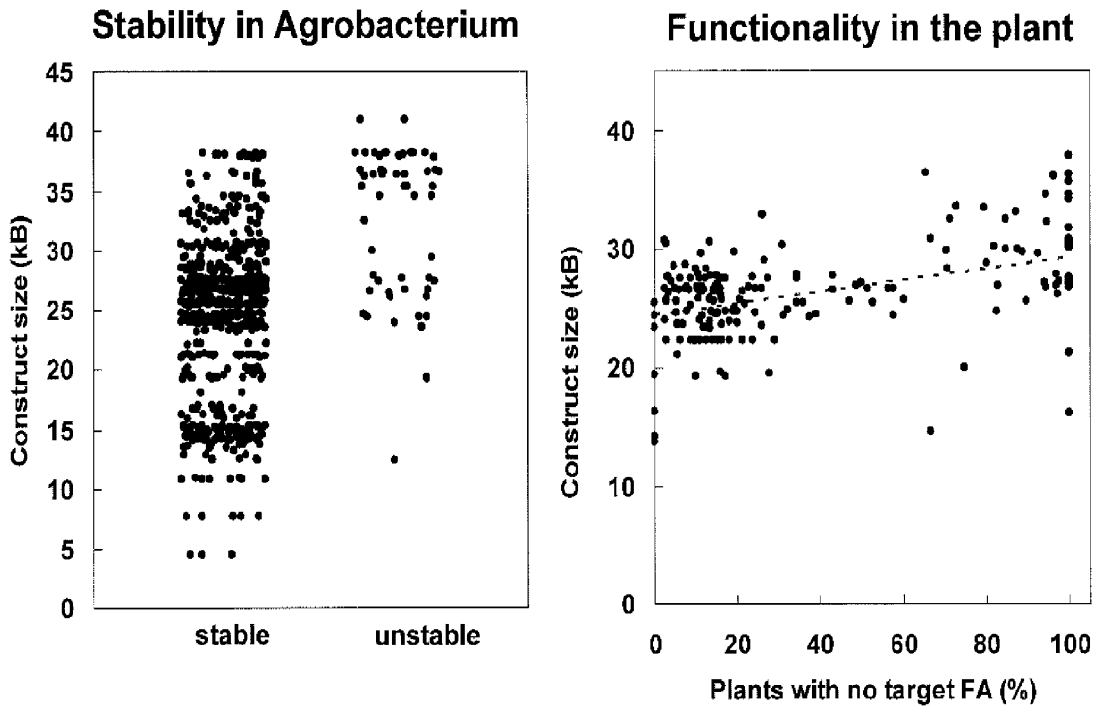


Figure 4: Stabiliy of binary plant expression plasmids containing the ColE1/pVS1 origin of replication for plasmid recplication in *E.coli*/Agrobacteria.Left Panel: Stability in *Agrobacterium* cells by isolating plasmid DNA from *Agrobacterium* cutures prior to usage of this culture for plant transformation, and subjecting the plasmid DNA to a restriction digest. An unexpected restriction pattern indicates disintegration/instability of the plasmid either in *E.coli* or in *Agrobacterium*. Right panel: Under the assumption at least one intact T-DNA from LB to RB was integrated into the plant genome during the transformation process most plants obtained via transformation of a given plasmid are expected to reach the desired trait encoded by the plasmid (here: production of novel fatty acids (FA) in the seeds). The decrease in the percentage of such 'functional' plants indicates instability either in Agrobacteria or during the transfer process into the plant or during the integration process into the genome. As can be seen, the proportion of non functional plants goes sharply up for plasmids above 25,000bp size when ColE1/pVS1 containing plasmids are used.

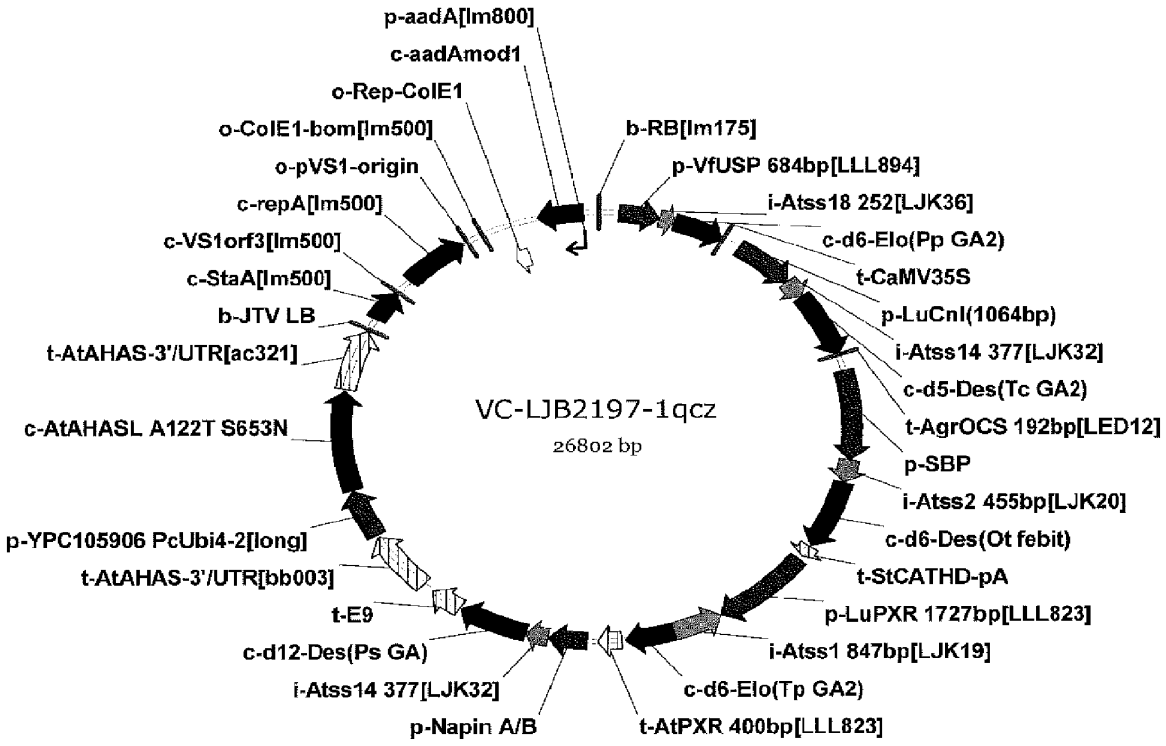


Figure 5: Plasmid map of VC-LJB2197-1qcz indicating the position of genetic elements listed in table 1

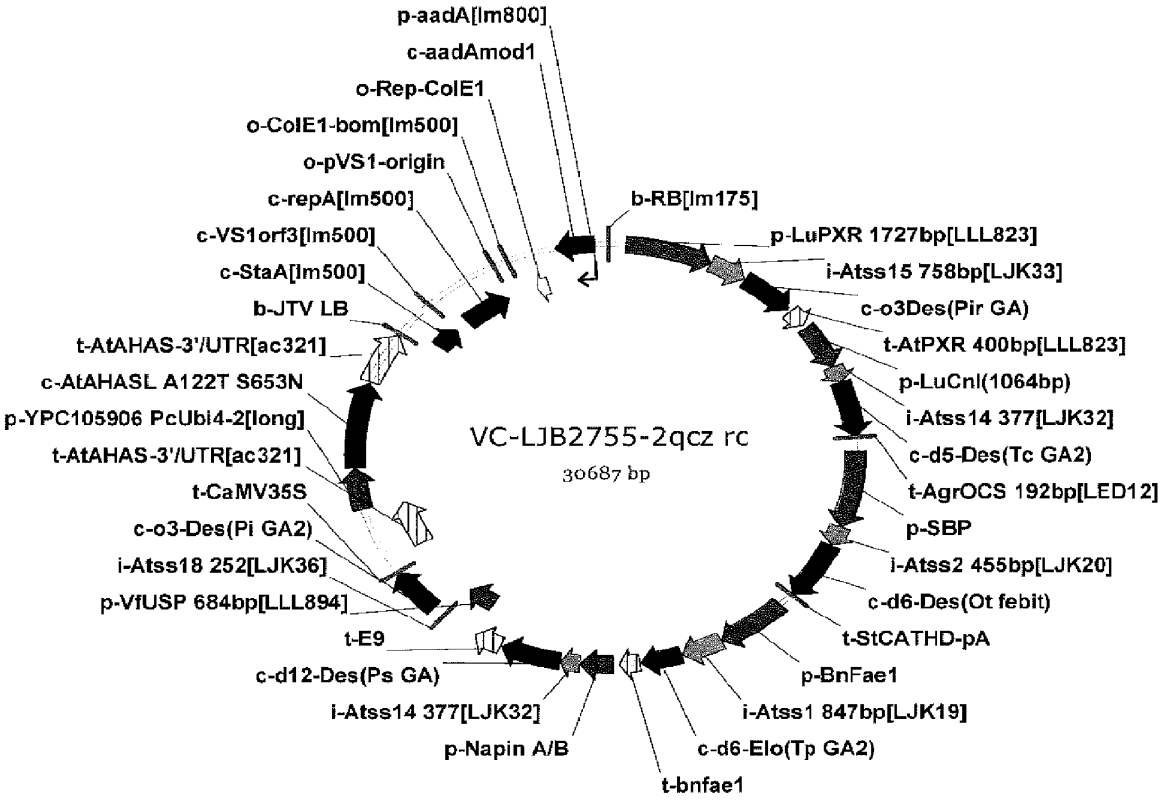


Figure 6: Plasmid map of VC-LJB2755-2qcz rc indicating the position of genetic elements listed in table 2

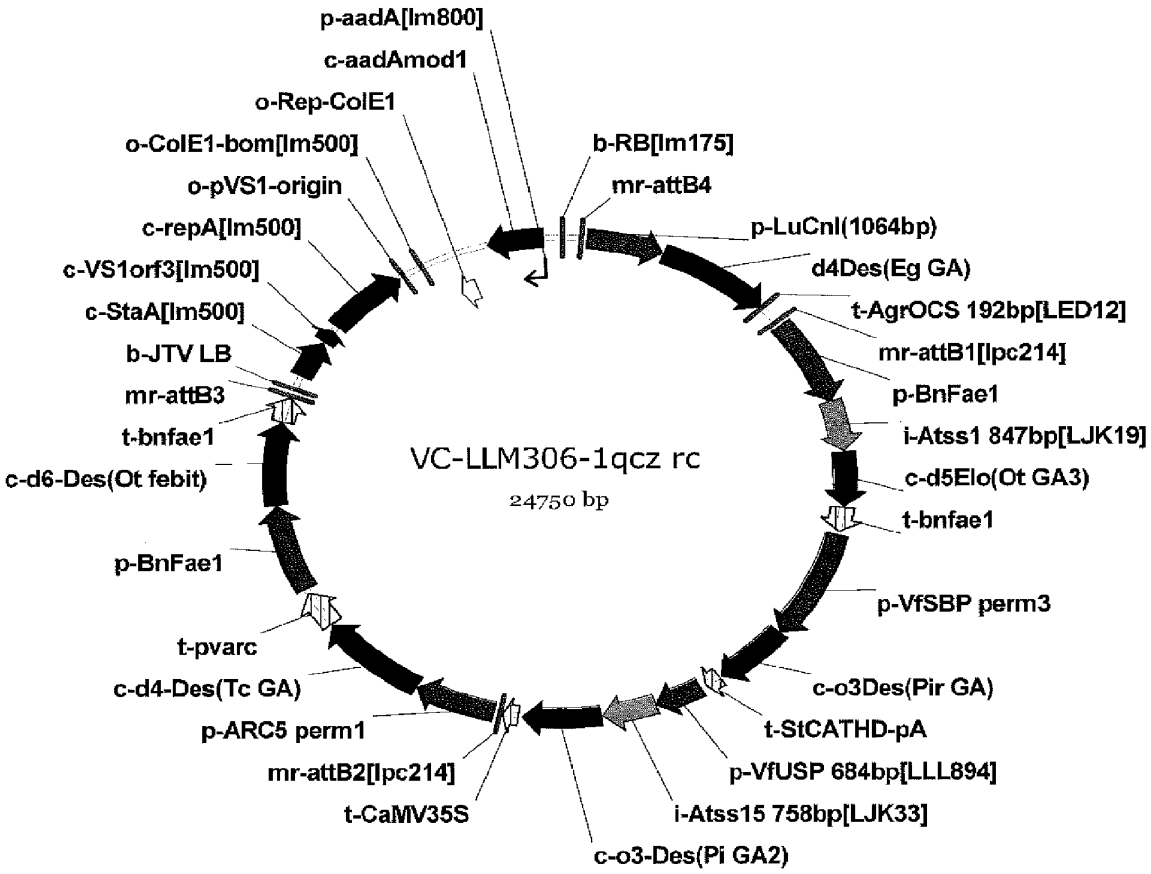


Figure 7: Plasmid map of VC-LLM306-1qcz rc indicating the position of genetic elements listed in table 3

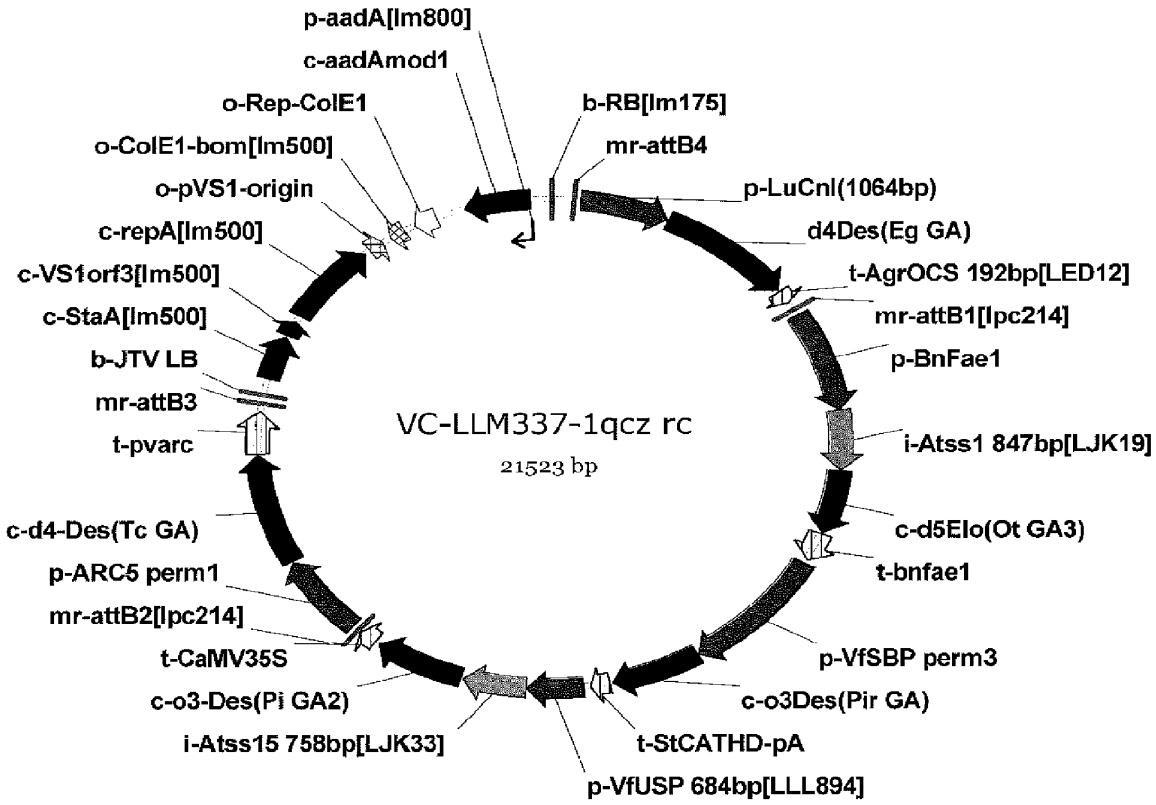


Figure 8: Plasmid map of VC-LLM337-1qcz rc indicating the position of genetic elements listed in table 4

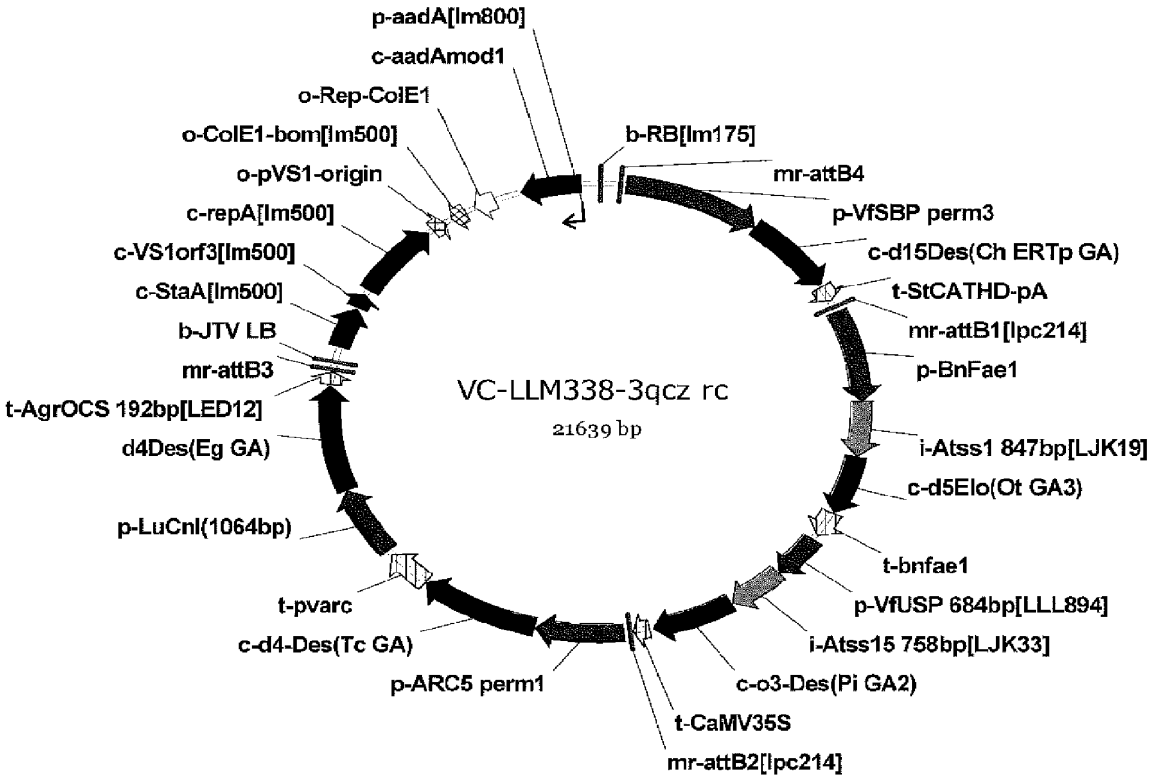


Figure 9: Plasmid map of VC-LLM338-3qcz rc indicating the position of genetic elements listed in table 5

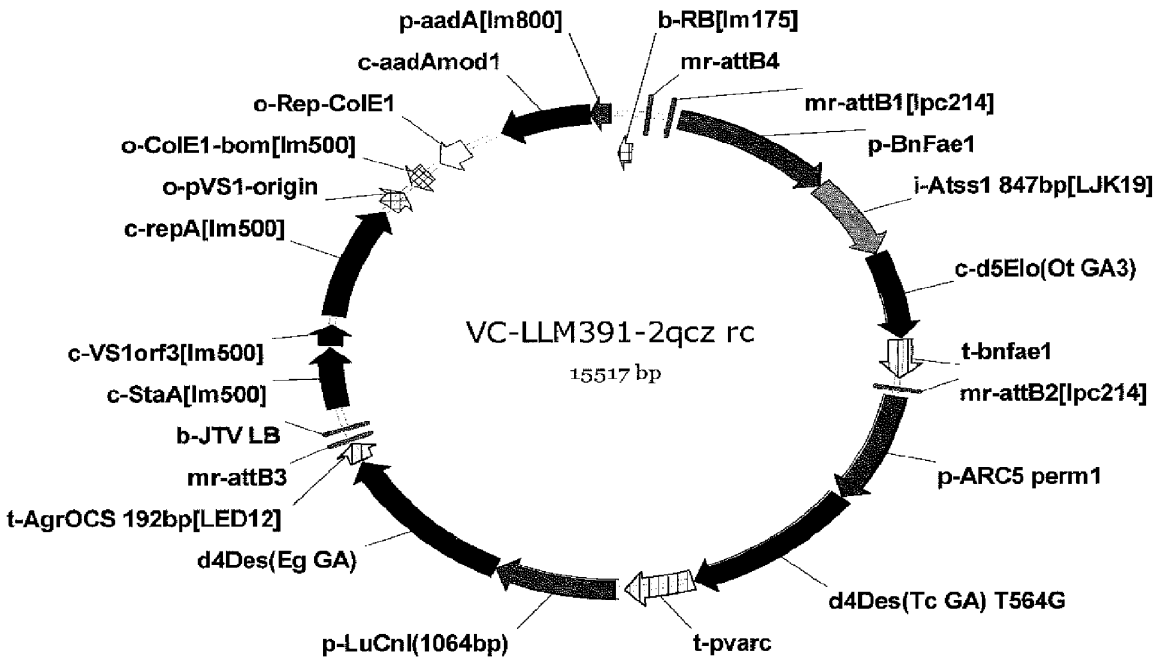


Figure 10: Plasmid map of VC-LLM391-2qcz rc indicating the position of genetic elements listed in table 6

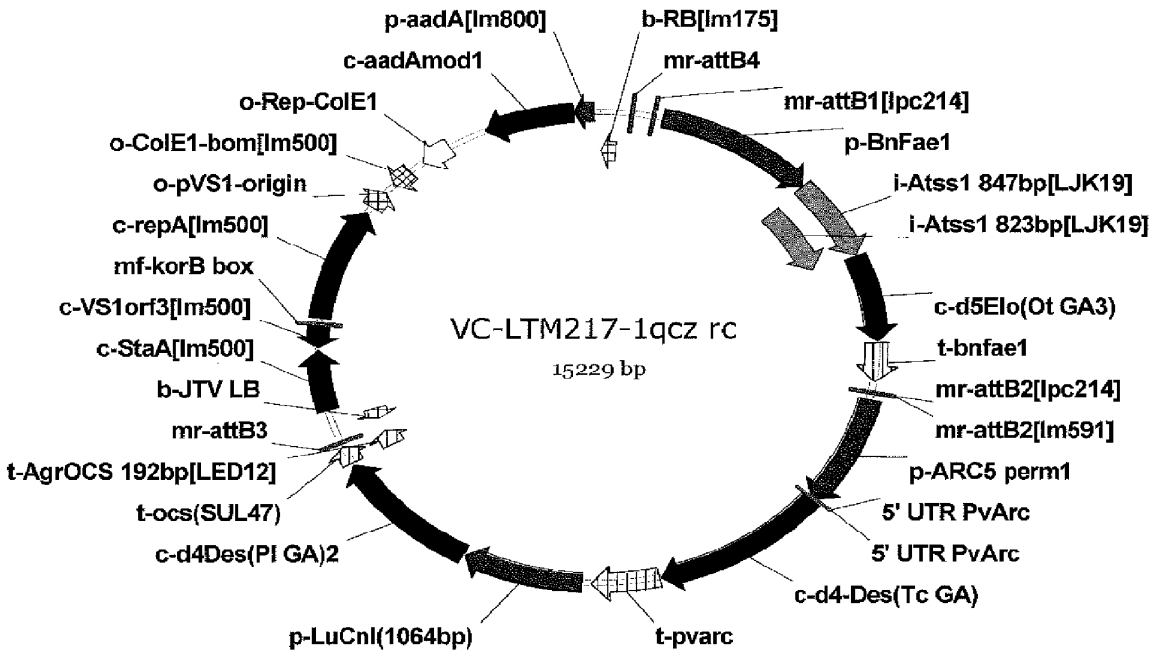


Figure 11: Plasmid map of VC-LTM217-1qcz rc indicating the position of genetic elements listed in table 7

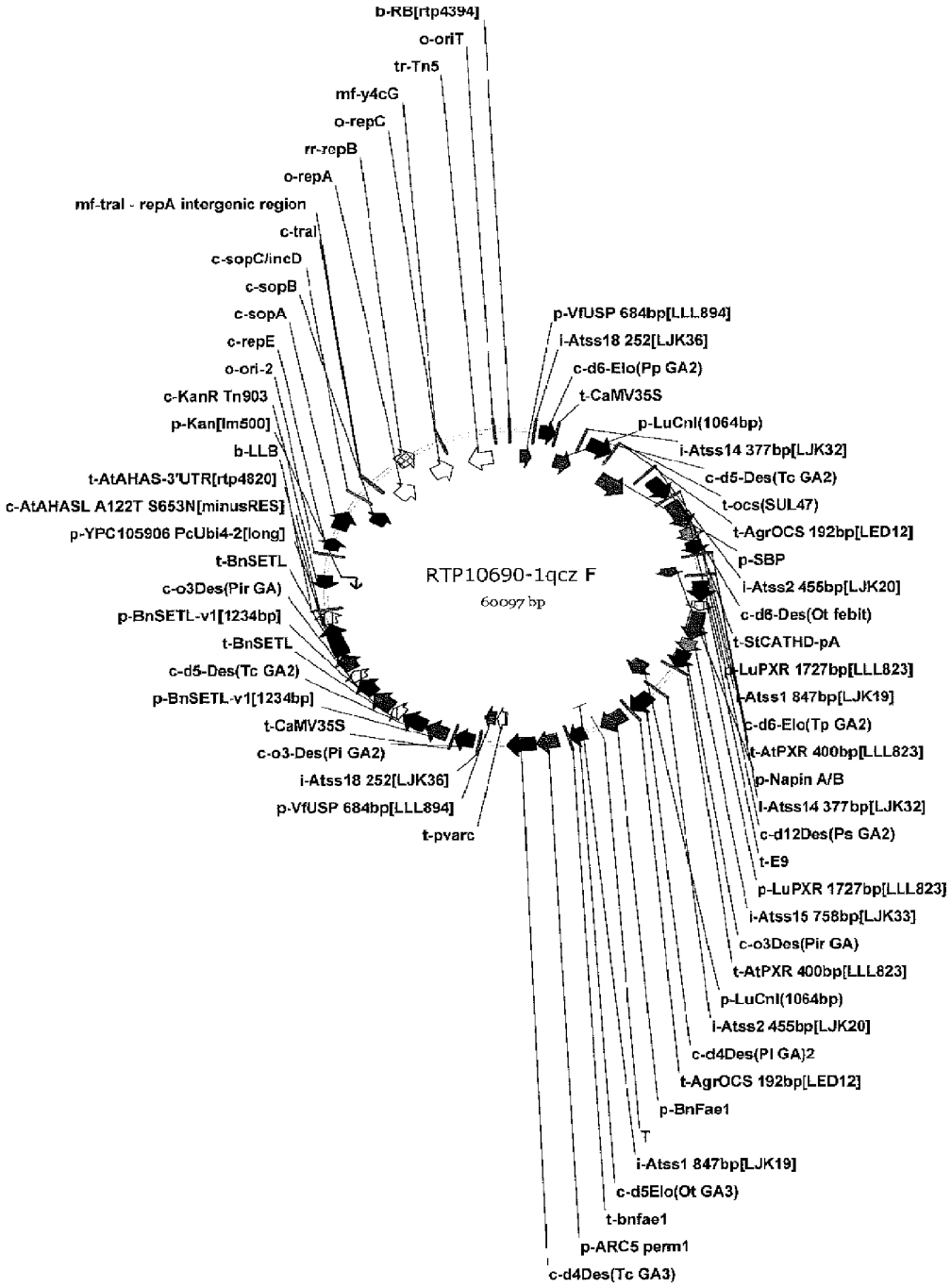


Figure 12: Plasmid map of RTP10690-1qcz_F indicating the position of genetic elements listed in table 8

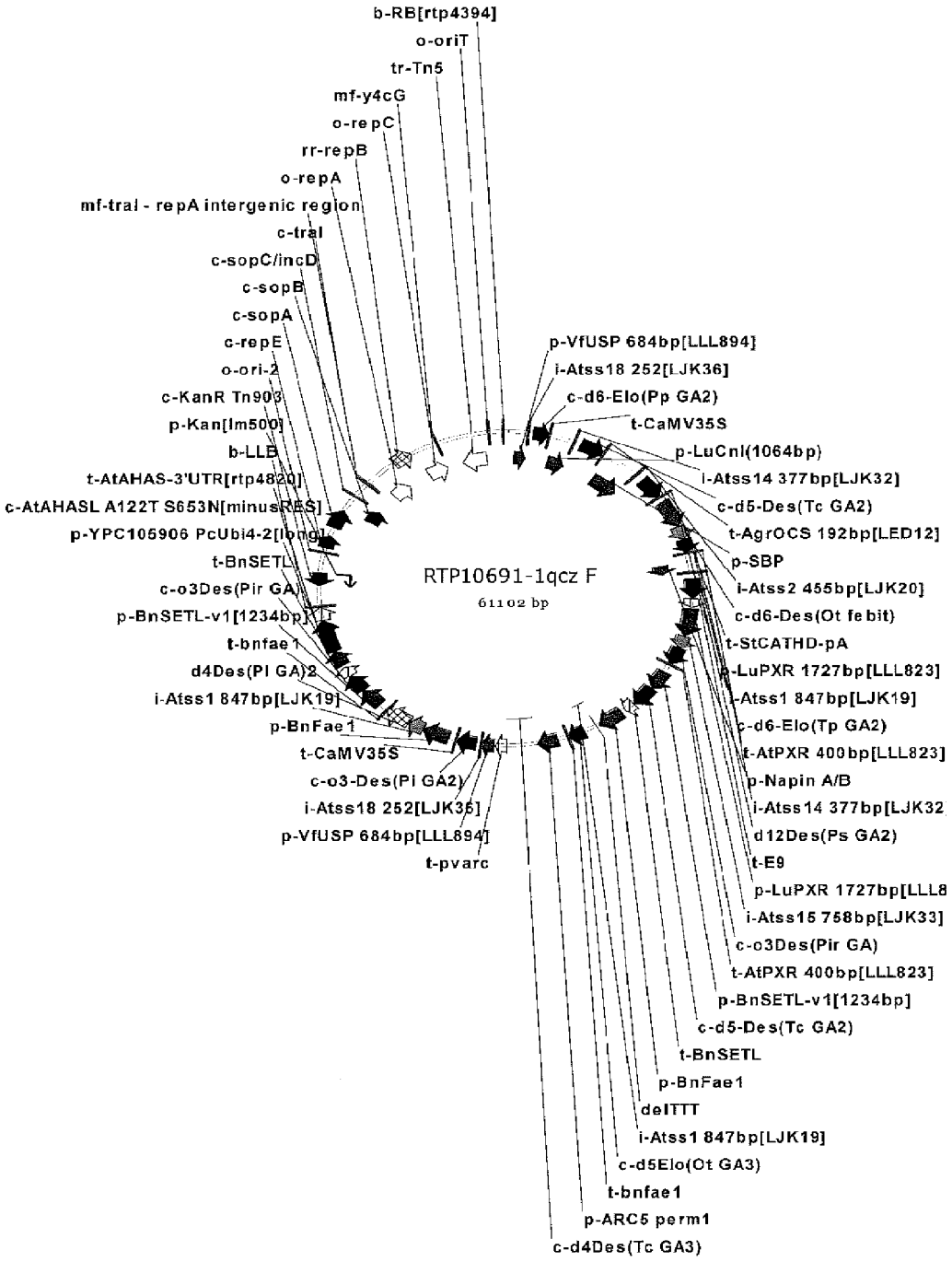


Figure 13: Plasmid map of RTP10691-2qcz indicating the position of genetic elements listed in table 9

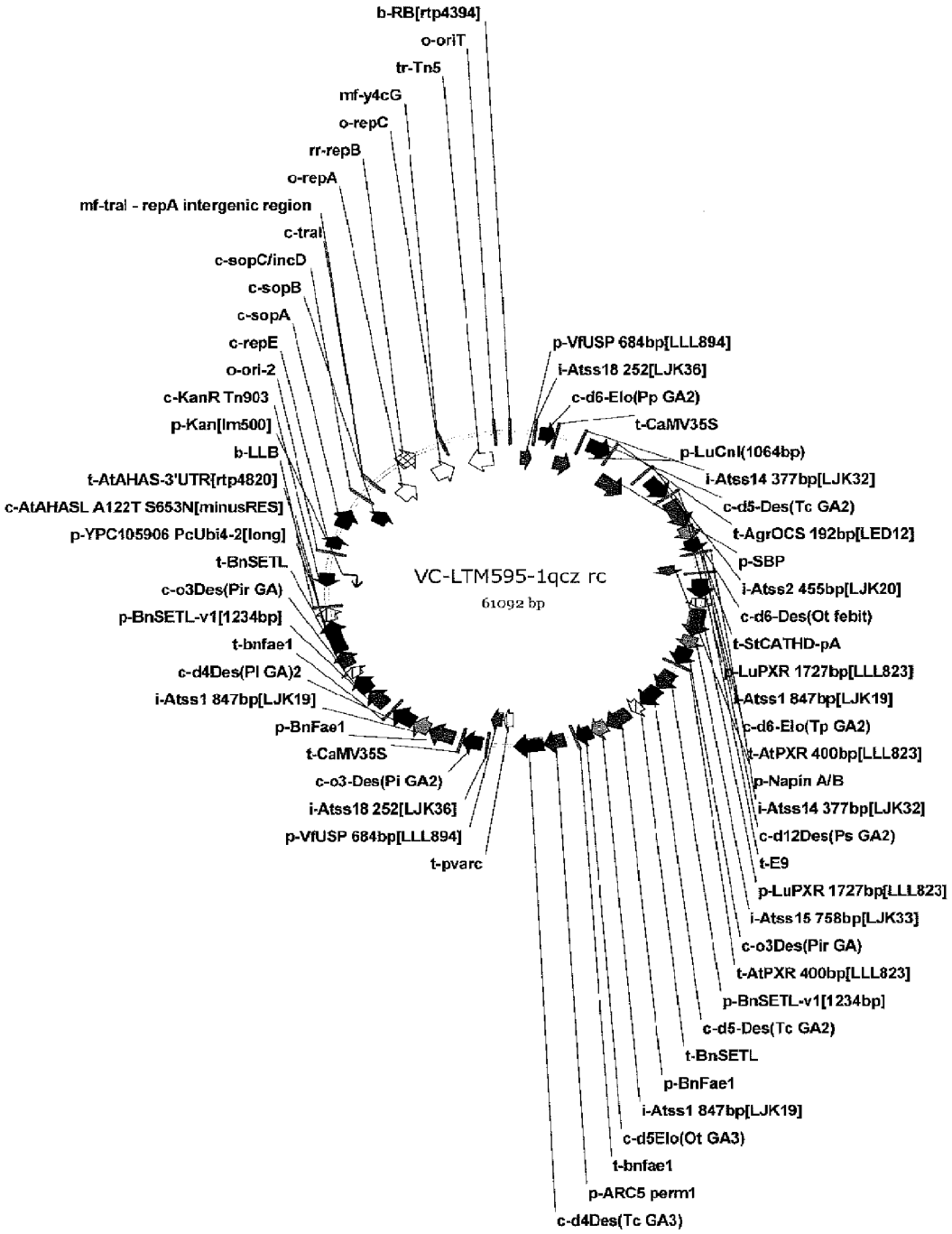


Figure 14: Plasmid map of LTM595-1qcz rc indicating the position of genetic elements listed in table 10

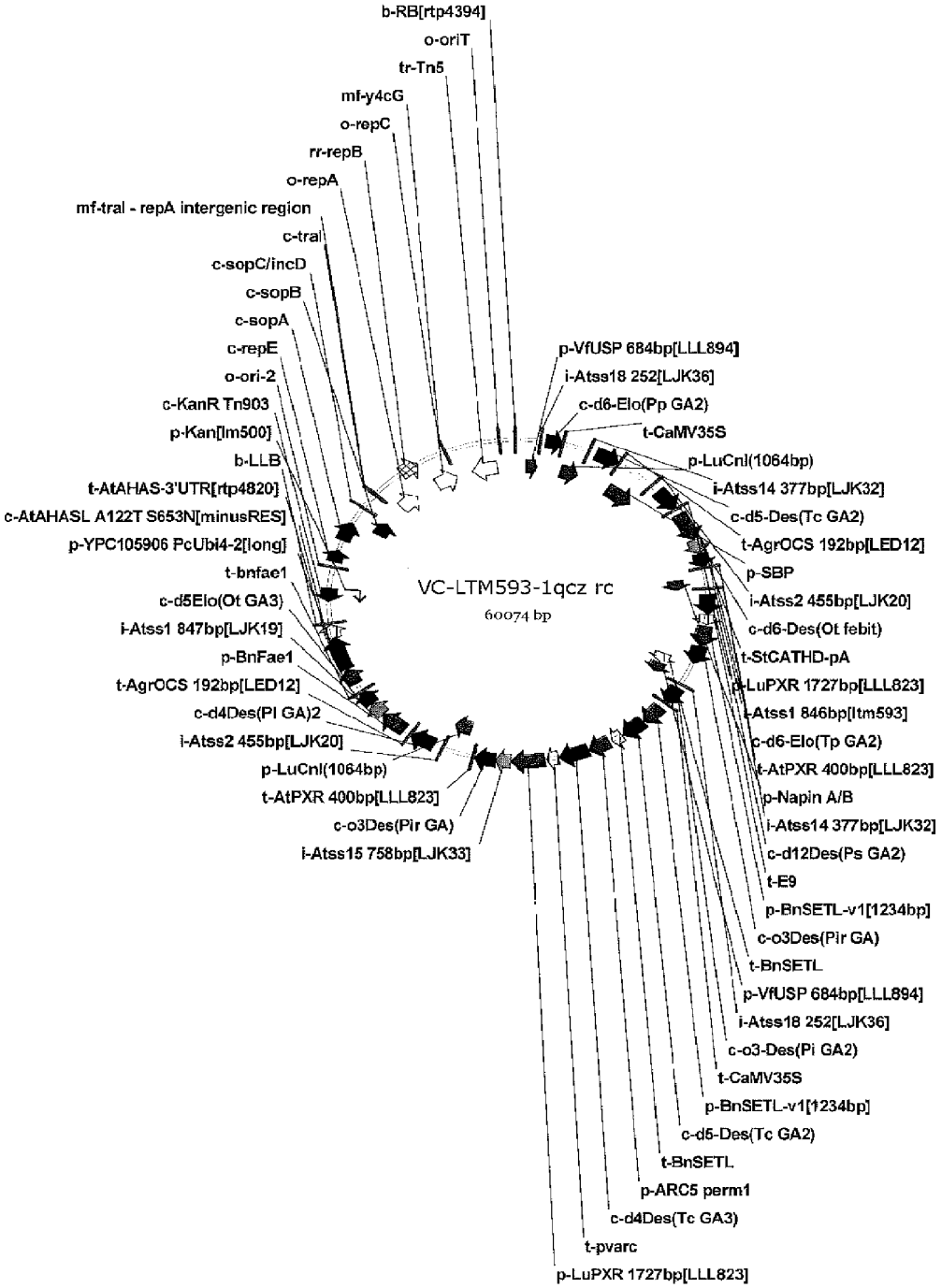


Figure 15: Plasmid map of LTM593-1qcz rc indicating the position of genetic elements listed in table 11

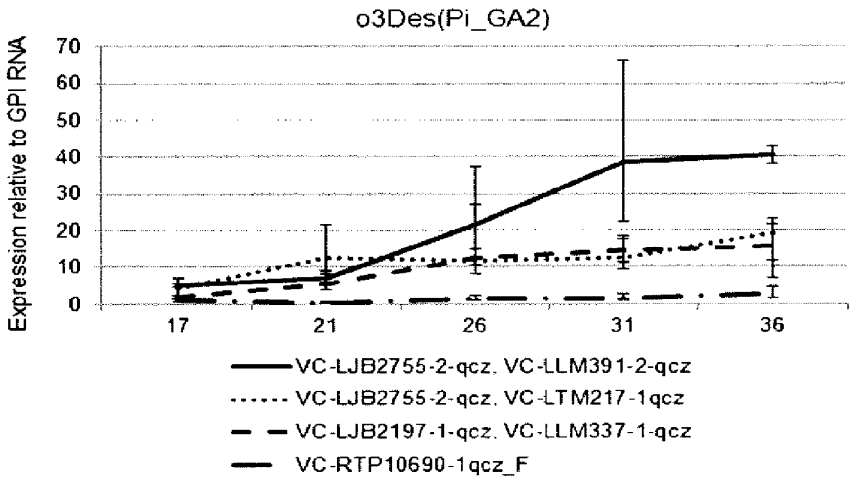


Figure 16: Comparative transcript analysis o3Des(Pi_GA2) driven by the VfUSP promoter during seed development of single copy event of four different construct combinations.

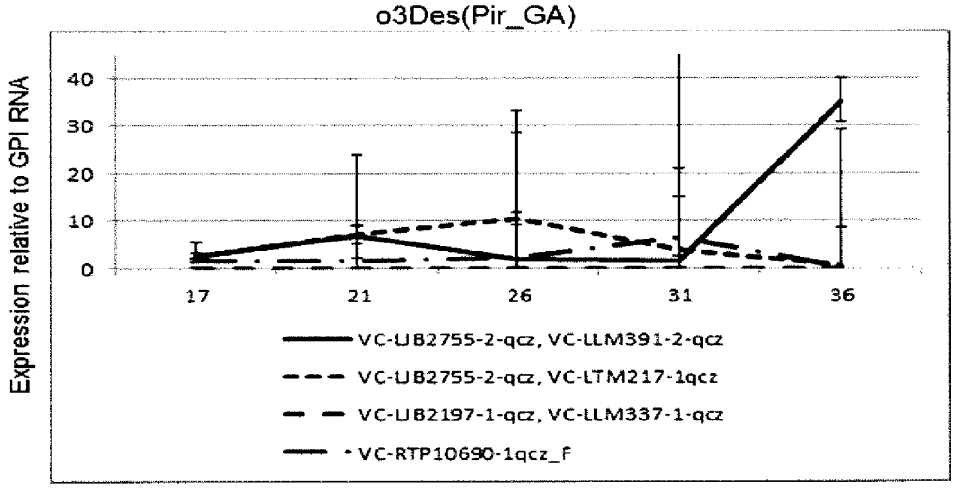


Figure 17: Comparative transcript analysis of o3Des(Pir_GA) during seed development of single copy event of four different construct combinations. In VC-LJB2755-2qcz and VC-RTP10690-1qcz_F the gene was driven by the LuConlinin promoter while in VC-LLM337-1qcz rc the gene was driven by the VfUSP promoter and was expressed at a lower level than the LuConlinin o3Des(Pir_GA) combination.

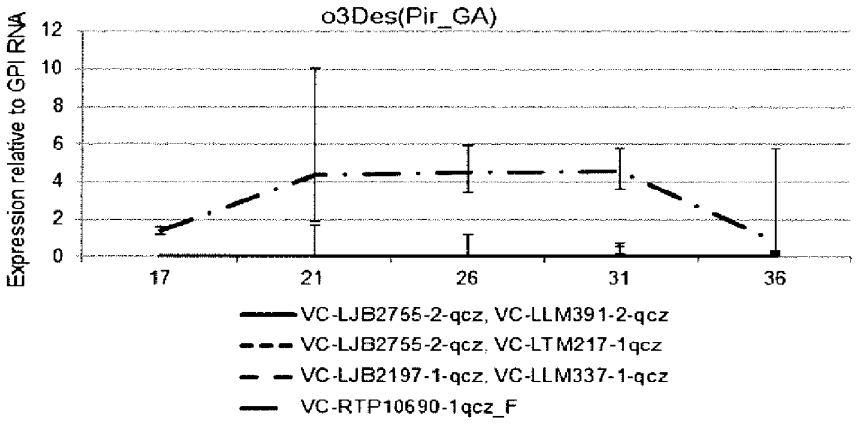


Figure 18: Comparative transcript analysis of o3Des(Pir_GA) driven by the BnSETL promoter during seed development of single copy event of VC-RTP10690-1qcz_F.

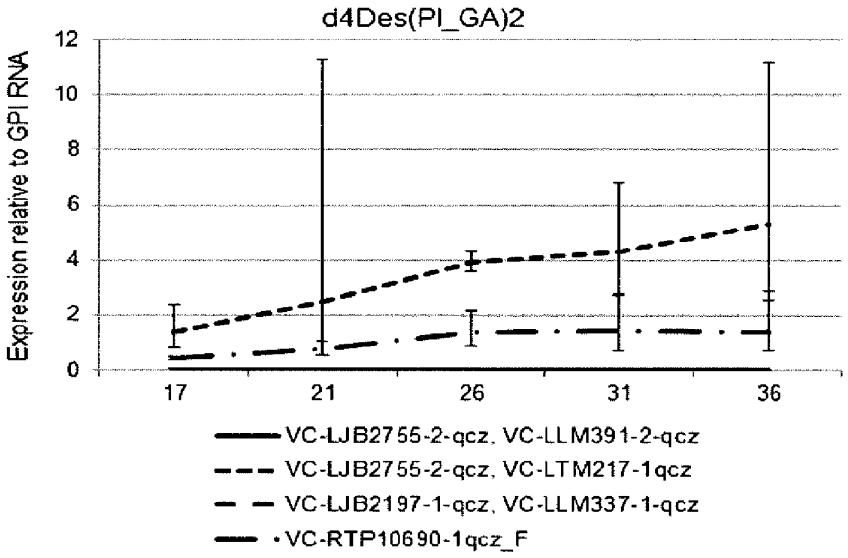


Figure 19: Comparative transcript analysis of d4Des(Pl_GA)2 driven by the LuConlinin promoter during seed development of single copy event from VC-RTP10690-1qcz_F and VC-LTM217-1qcz rc, which was present with VC-LJB2755-1qcz. The other constructs lacked this particular d4Des.

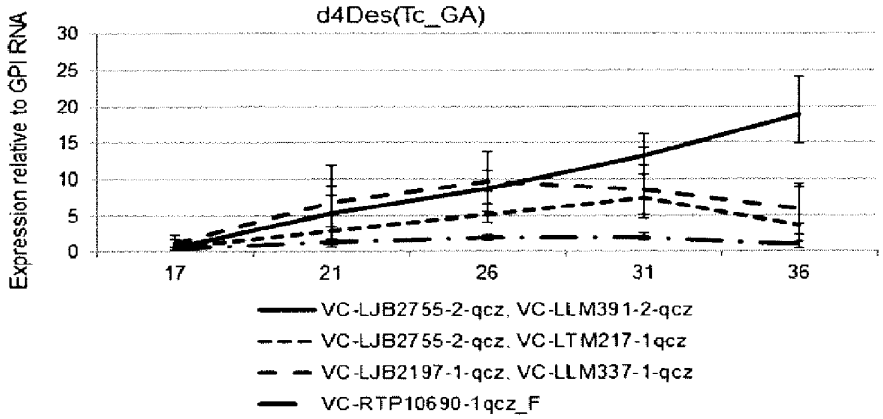


Figure 20: Comparative transcript analysis of d4Des(Tc_GA) driven by the ARC5 promoter during seed development of single copy event of four different construct combinations.

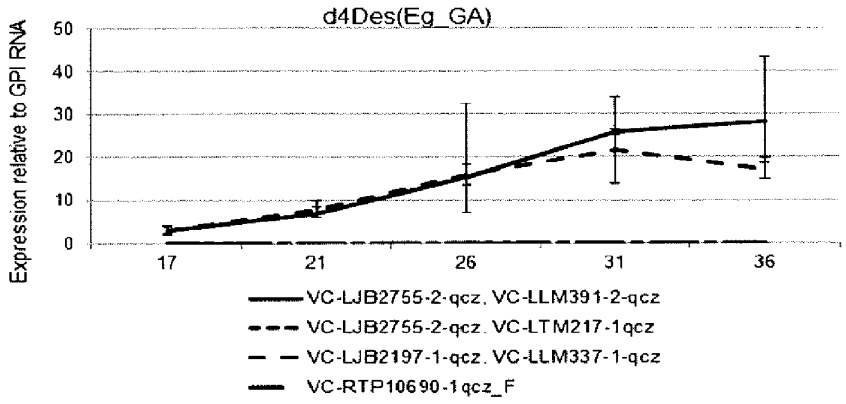


Figure 21: Comparative transcript analysis of d4Des(Eg_GA) driven by the LuConlinin promoter during seed development of single copy event of two different construct combinations; VC-LJB2755-2qcz, VC-LLM391-2qcz rc and VC-LJB2197-1qcz, VC-LLM337-1qcz rc.

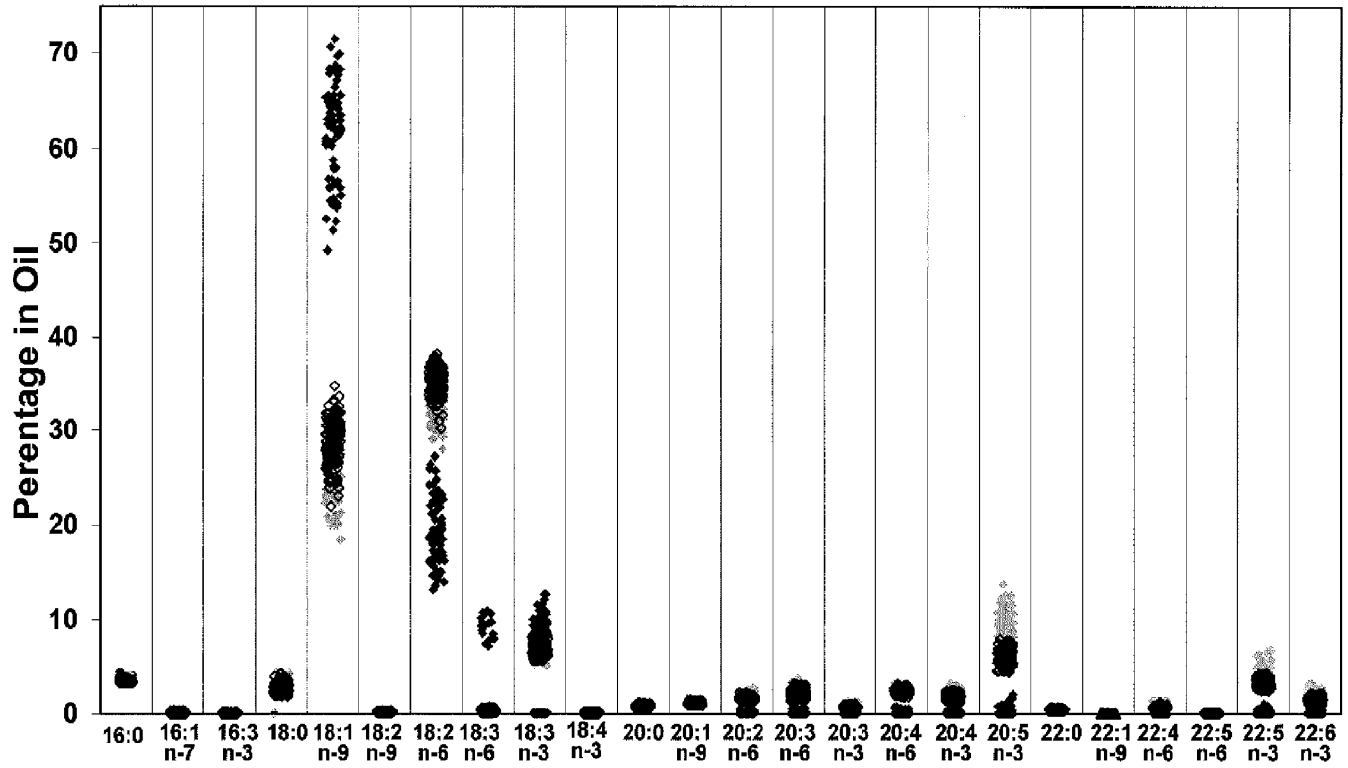


Figure 22: Half Kernel Analysis of segregating T1 seeds of Event LANPMZ. A total of 288 seedlings where analysed. 71 of those seedlings were found to produce no significant amount of VLC-PUFA (dark grey diamonds) while containing >49% Oleic acid and <28% Linoleic acid. 71 seed of 288 seed correspond to 24.65% of the total analysed seed. All remaining seed were capable of producing DHA, indicating the presence of both T-DNA from construct VC-LJB2197-1qcz and VC-LLM337-1qcz rc. Among those seeds producing DHA, one can discriminate a group of 146 seeds showing medium VLC-PUFA levels (open diamonds), and a group of 71 seed showing high VLC-PUFA levels (light grey diamonds). The ratios of these three groups is 71:146:71, which corresponds to the Medelain 1:2:1 ratio (NULL:HETEROZYGOUS:HOMOZYGOUS) expected for a phenotype when all gene conveying this phenotype (in this case the two T-DNAs of plasmid VC-LJB2197-1qcz and VC-LLM337-1qcz rc) integrated into one locus in the genome.

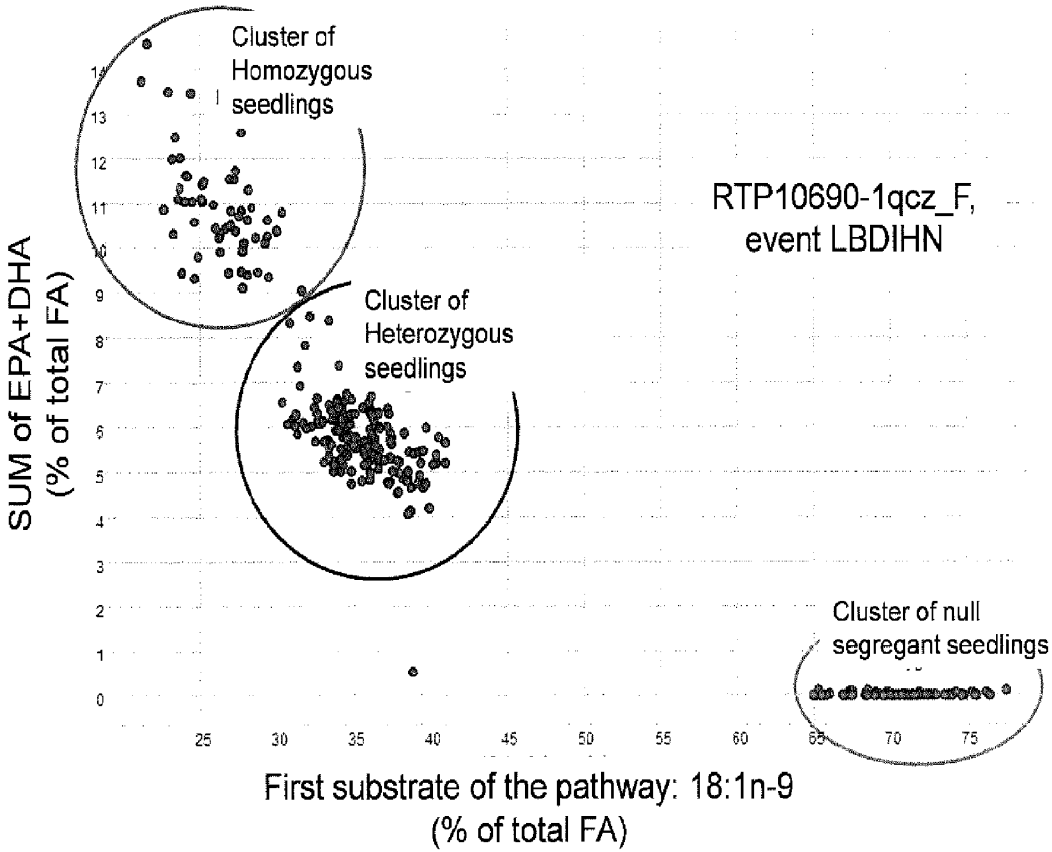


Figure 23: Half Kernel Analysis of segregating T1 seeds of Event LBDIHN. A total of 288 seedlings where analysed. The levels of first substrate fatty acid of the pathway was plotted on the x-axis, the levels of the sum of two products of the pathways (EPA+DHA) was plotted on the y-axis. One can clearly see three clusters, where the ratio of the number of seeds in the these three clusters was 1:2:1 (Homozygous:Heterozygous:Null segregant). This segregation of the phenotype according to the first Mendelian law demonstrates a single locus insertion of the T-DNA of construct RTP10690-1qcz_F into the genome of *B.napus cv Kumily*.

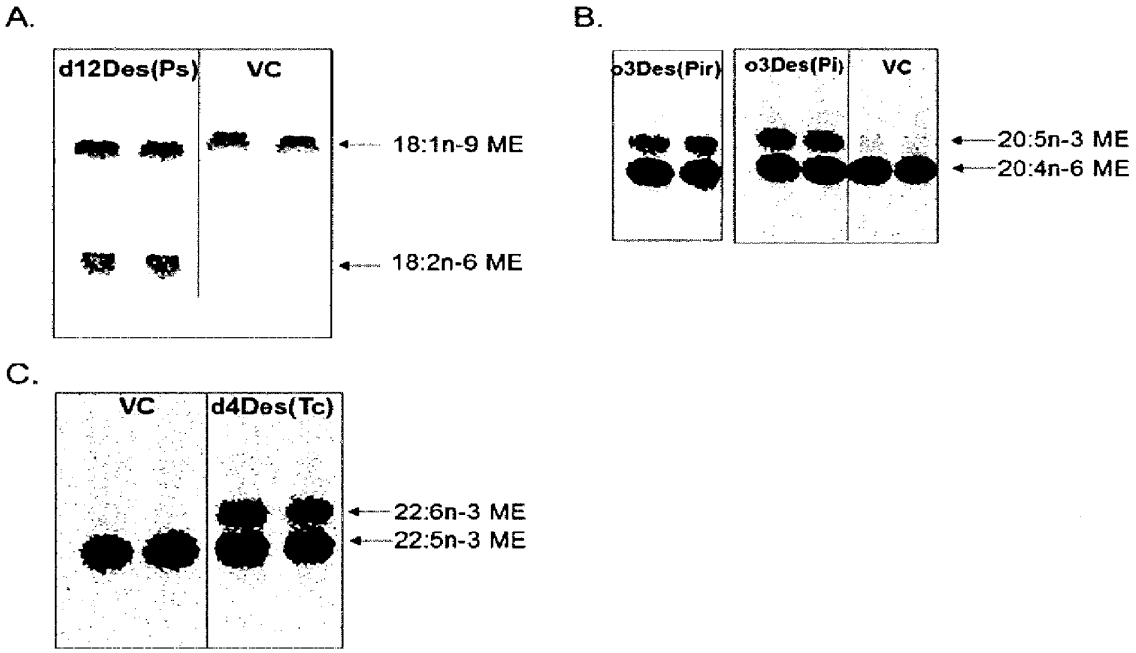


Figure 24: Examples of Desaturase Enzyme Activity Heterologously Expressed in Yeast. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the c-d12Des(Ps_GA) protein relative to microsomes isolated from a control strain containing an empty vector (VC). In panel B Omega-3 Desaturase activities, c-o3Des(Pir_GA) and c-o3Des(Pi_GA2), activities were demonstrated by comparison of enzyme activity from yeast microsomes isolated from strains expressing c-o3Des(Pir_GA) protein, c-o3Des(Pi_GA2) protein or an empty vector (VC) control. In panel C Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d4Des(Tc_GA) protein relative to microsomes isolated from a control strain containing an empty vector (VC).

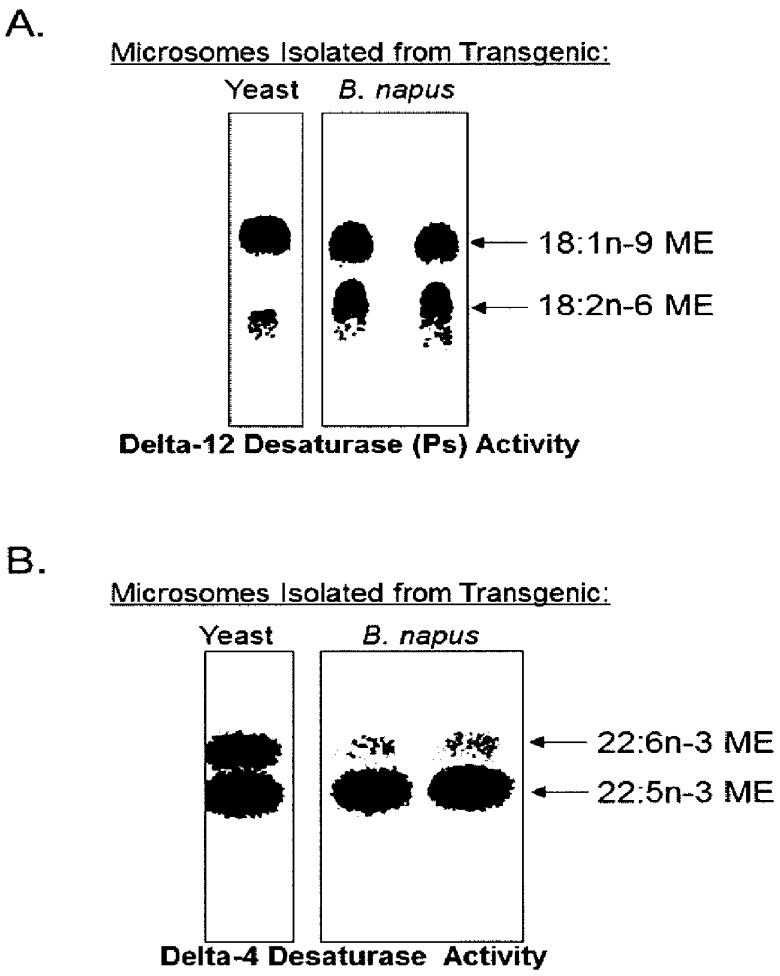
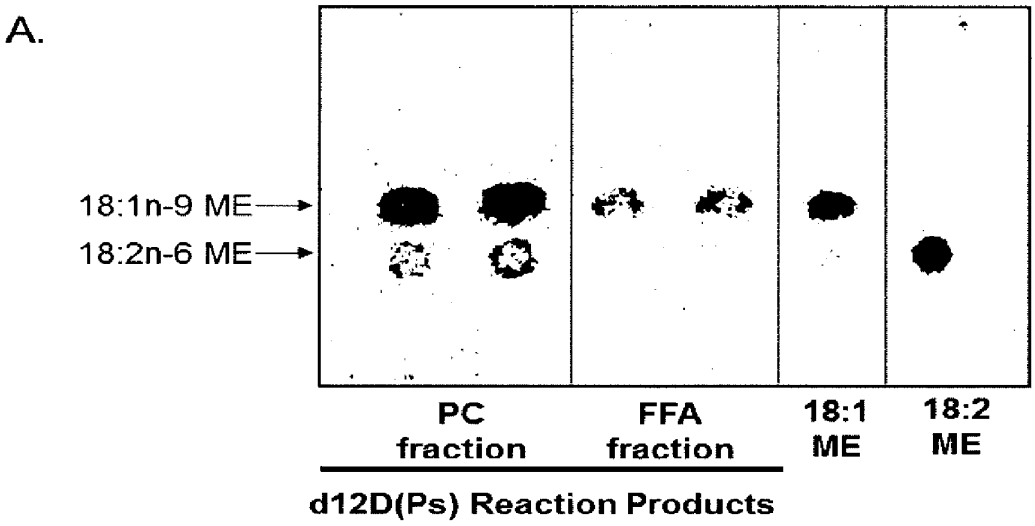
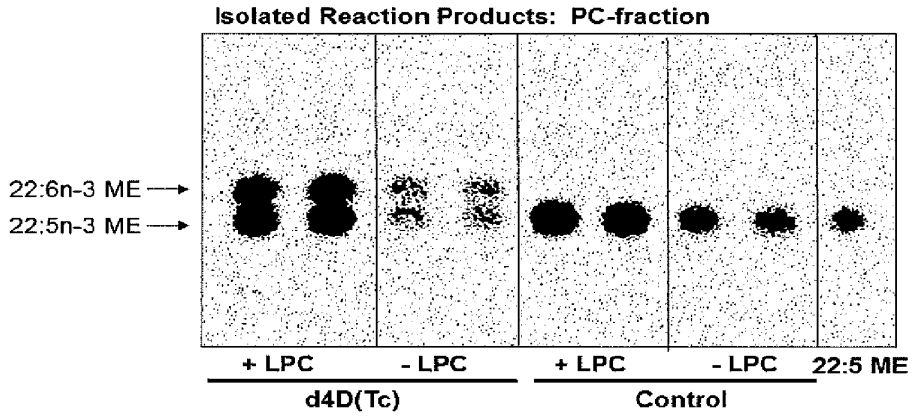


Figure 25: Examples of Desaturase Enzyme Activity in transgenic *Brassica napus*. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d12Des(Ps_GA) protein relative to microsomes isolated from transgenic *B. napus* containing the *d12Des(Ps_GA2)* gene. In panel B Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), and Delta-4 Desaturase (Pl) activities were demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d4Des(Tc_GA) protein relative to microsomes isolated from transgenic *B. napus* containing the *d4Des(Tc_GA3)* and *d4Des(Pl_GA)2* genes.

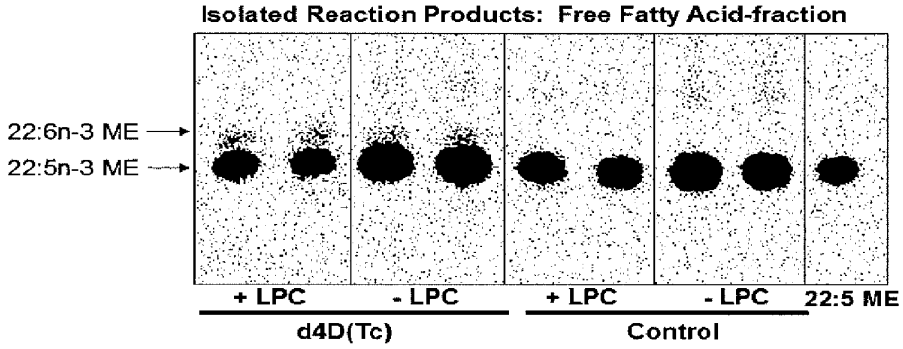
A.


18:1n-9 ME
18:2n-6 ME
PC fraction
FFA fraction
18:1 ME
18:2 ME
d12D(Ps) Reaction Products

B.


Isolated Reaction Products: PC-fraction
22:6n-3 ME
22:5n-3 ME
+ LPC
- LPC
+ LPC
- LPC
22:5 ME
d4D(Tc)
Control

C.


Isolated Reaction Products: Free Fatty Acid-fraction
22:6n-3 ME
22:5n-3 ME
+ LPC
- LPC
+ LPC
- LPC
22:5 ME
d4D(Tc)
Control

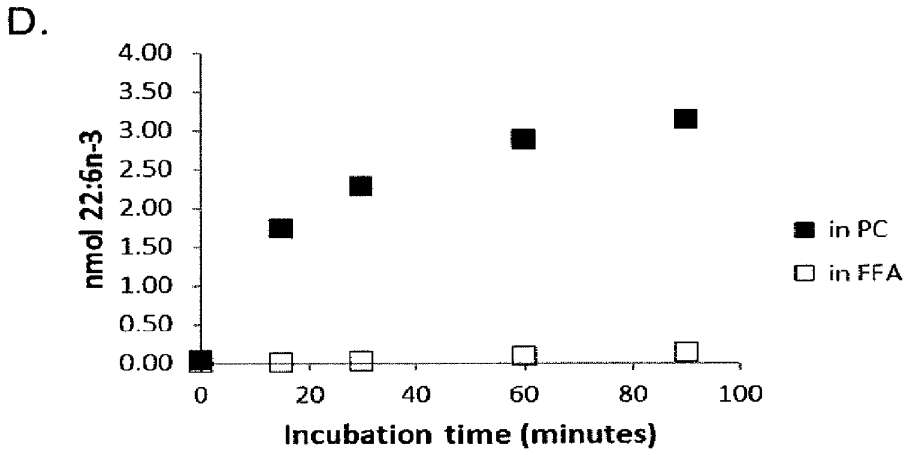
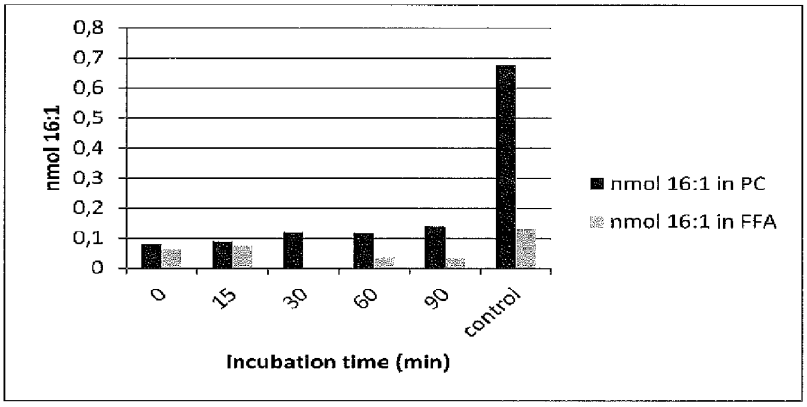
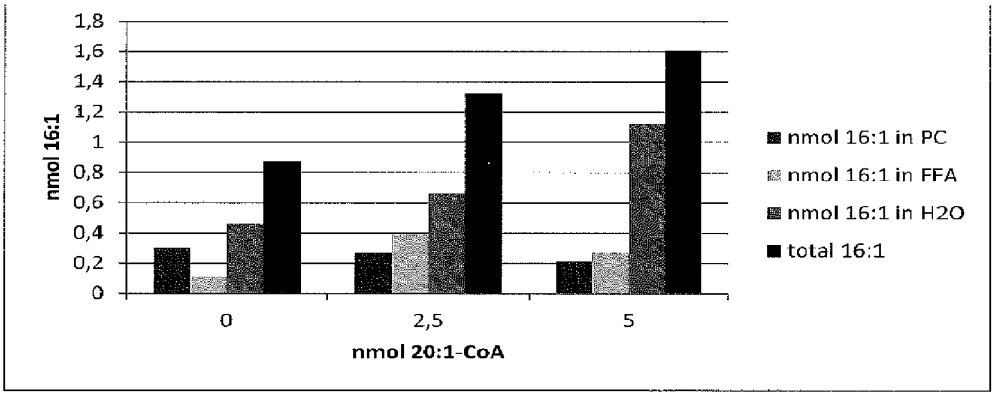


Figure 26: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-lipid substrates. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions containing microsomes obtained from a yeast strain expressing the protein of interest, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-12 Desaturase (Ps), c-d12Des(Ps_GA), desaturated enzyme products were only detected in the phosphatidylcholine fraction indicating the enzyme was specific for an acyl-lipid substrate. In panel B and panel C Delta-4 Desaturase (Tc), c-d4Des(Tc_GA), desaturated enzyme products were detected in the phosphatidylcholine fraction indicating the enzyme was specific for an acyl-lipid substrate. In panel D was a time-course demonstrates of the Delta-4 Desaturase (Tc), c-d4Des(Tc_GA).

A.


nmol 16:1
0,8
0,7
0,6
0,5
0,4
0,3
0,2
0,1
0
0
15
30
60
90
control
Incubation time (min)
nmol 16:1 in PC
nmol 16:1 in FFA

B.


nmol 16:1
1,8
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
0
2,5
5
nmol 20:1-CoA
nmol 16:1 in PC
nmol 16:1 in FFA
nmol 16:1 in H2O
total 16:1

C.

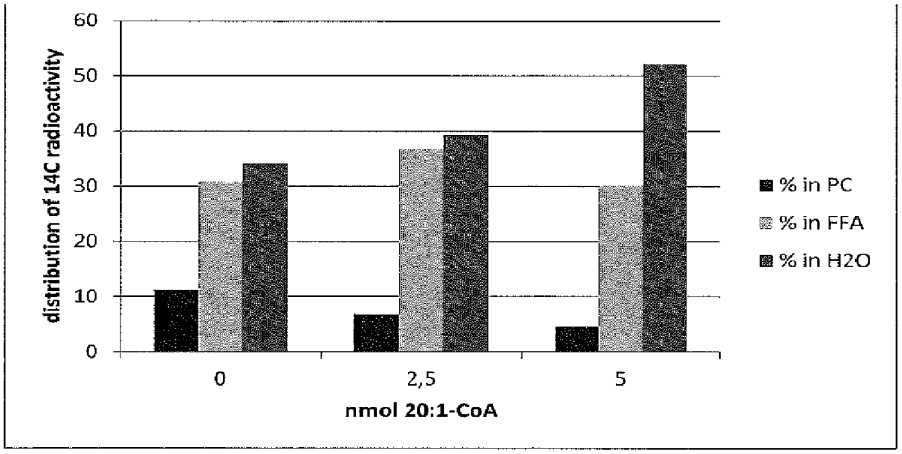

distribution of 14C radioactivity
60
50
40
30
20
10
0
0
2,5
5
nmol 20:1-CoA
% in PC
% in FFA
% in H2O

Figure 27: Examples of Desaturase Enzyme Reactions Showing Specificity for Acyl-CoA substrates. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions containing microsomes obtained from a yeast strain expressing the protein of interest, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A PC was in situ labeled with substrate according to the method for determining lipid linked desaturation. Delta-9 Desaturase (Sc), d9D(Sc), desaturated enzyme products were very low in the phosphatidylcholine fraction, except for in the control reaction (none in situ labeled PC), indicating the enzyme cannot desaturate an acyl-lipid substrate. In panel B and C the incubation was done according to the method for determining acyl-CoA linked desaturation. In panel B the amount of radioactivity in the acyl-CoA fraction (MeOH/H2O-phase) was increasing when 20:1-CoA was added to the assay. This indicates that the added 20:1-CoA was competing with the radioactive substrate in formation of PC and free fatty acids. In panel C the amount of desaturated enzyme products was increased in the acyl-CoA fraction when 20:1-CoA was added to the assay, indicating that the desaturation was acyl-CoA linked.

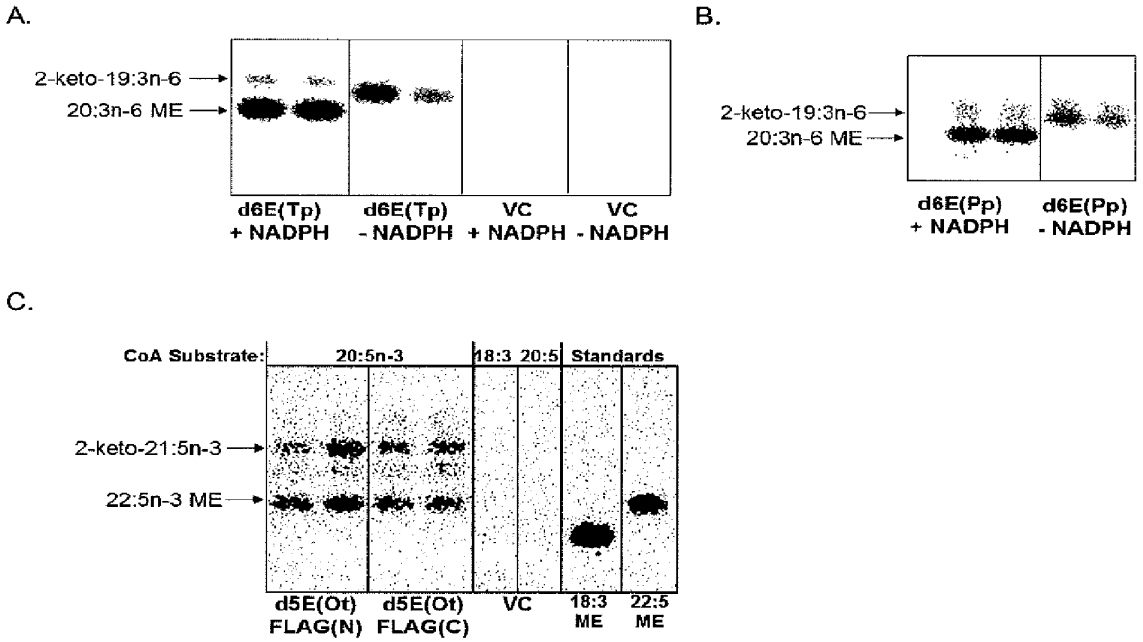


Figure 28: Examples of Elongase Enzyme Activity Heterologously Expressed in Yeast. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. All FAME's shown had similar Rf's as authentic standards. In the absence of [^{14}C]malonyl-CoA no radioactive fatty acids were observed in any of these elongase reactions. In panel A delta-6 elongase (Tp), c-d6Elo(Tp_GA2), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the c-d6Elo(Tp_GA2) protein relative to microsomes isolated from a control strain containing an empty vector (VC). In panel B, delta-6 elongase (Pp), c-d6Elo(Pp_GA2), was demonstrated by comparison of enzyme acitivity from yeast microsomes isolated from a strains expressing c-d6Elo(Pp_GA2) protein to microsomes isolated from a control strain containing an empty vector (VC), as shown in panel

A. In panel C, delta-5 elongase (Ot), c-d5Elo(Ot_GA3), activity was demonstrated by comparison of enzyme activity present in yeast microsomes isolated from a strain expressing the d6E(Ot) protein relative to microsomes isolated from a control strain containing an empty vector (VC).

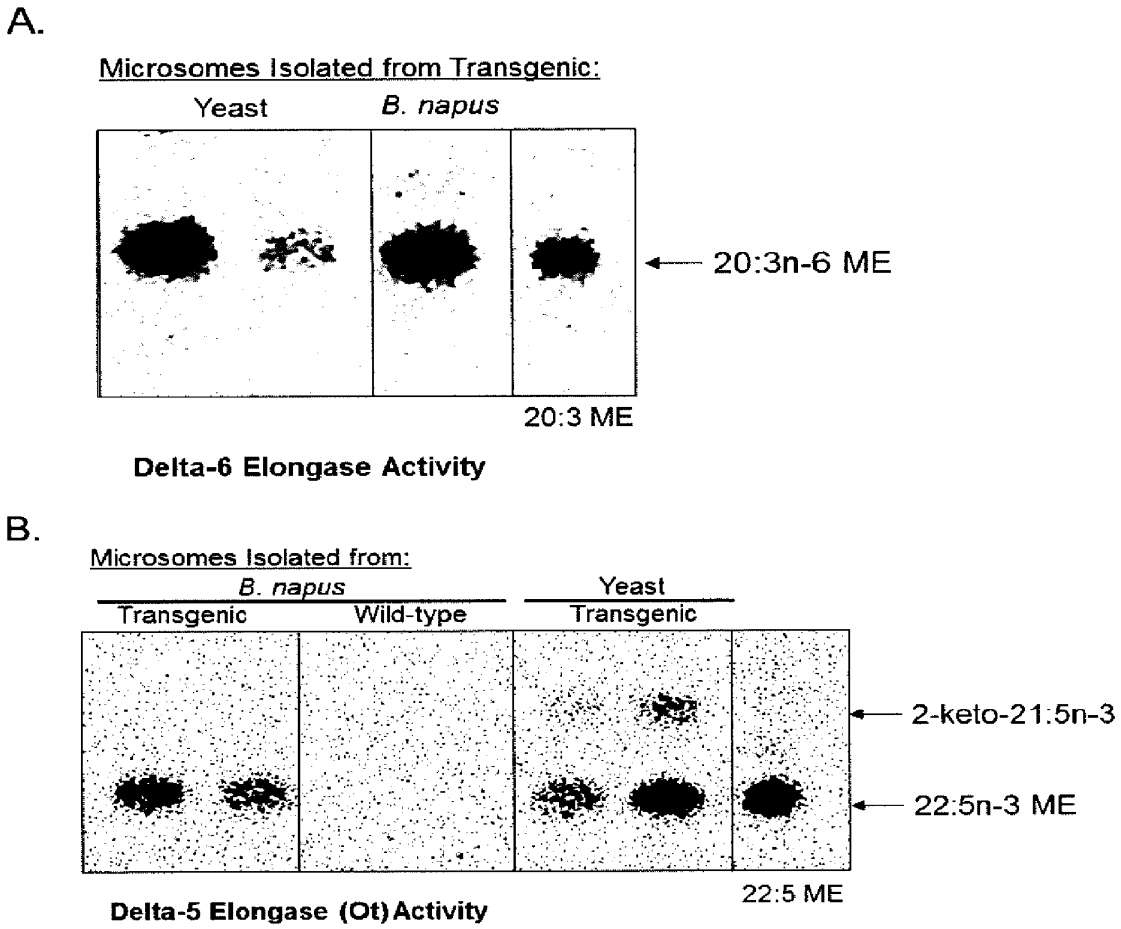


Figure 29: Examples of Elongase Activity in transgenic *Brassica napus*. [^{14}C]Fatty acid methyl esters (ME's) were isolated from the enzymatic reactions, resolved by TLC as described for each specific enzyme and detected by electronic autoradiography using Instant Imager. In panel A Delta-6 Elongase, activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the d6E(Pp protein relative to microsomes isolated from transgenic *B. Napus* containing the *d6Elo(Pp_GA2)* gene and the *d6Elo(Tp_GA2)*. In panel B the Delta-5 Elongase (Ot), c-d5Elo(Ot_GA3), activity was demonstrated by comparison of enzyme activity from yeast microsomes isolated from a strain expressing the c-d5Elo(Ot_GA3) protein relative to microsomes isolated from transgenic *B. Napus* containing the *d5Elo(Ot_GA3)* and a wild-type *B. napus* (control).

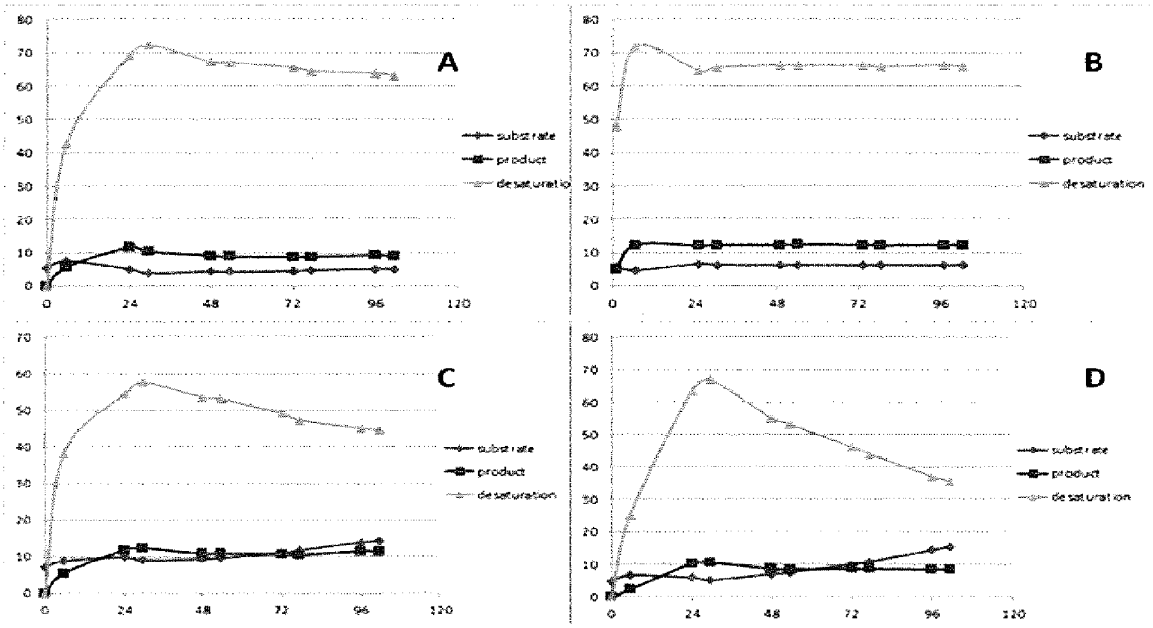


Figure 30: Time course optimization.Yeast cells expressing the c-d5Des(Tc_GA2) were fed with 0.25 mM HGLA and the production of ARA was determined by GC. Samples were collected starting immediately after feeding. In Panels A-D, Desaturation was represented as %Conversion vs Growth Time (hours) and Product and Substrate levesl are represented as %Total Fatty acid vs Growth time (hours). Panel A Samples supplied with HGLA immediately after induction. Panel B overnight induction (22hrs) before feeding. Panel C cultures supplied with 3X normal HGLA level Panel D. cultures supplied with normal rate of HGLA (0.25 mM) daily.

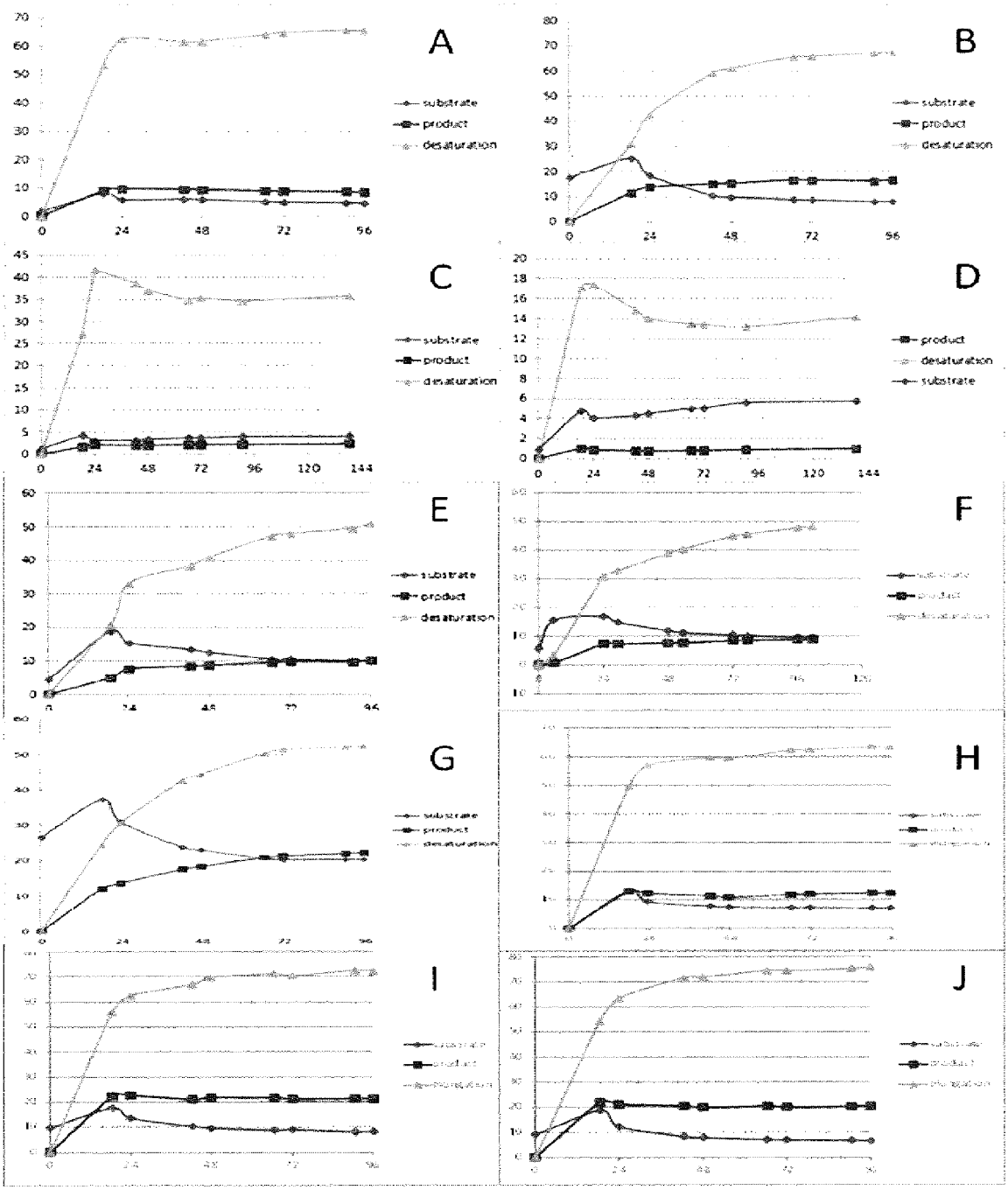


Figure 31: Representative time course graphs for all desaturases and elongases. Yeast cells expressing each enzyme were supplied with 0.25 mM of preferred fatty acid substrate, and fatty acid profiles were obtained by GC at the indicated time points. In Panels A-J, Desaturation and Elongation were represented as %Conversion vs Growth

Time (hours), and Product and Substrate levels were represented as %Total Fatty acid vs Growth time (hours).A. c-d5Des(Tc_GA2) + **HGLA** B. c-d6Des(Ot_fobit) + **ALA** C. c-d4Des(Pl_GA)2 + **DTA** D. c-d4Des(Tc_GA) + **DTA** E. c-o3Des(Pir_GA) + **ARA** F. c-o3Des(Pi_GA2) + **ARA** G. c-d12Des(Ps_GA) + **OA** H. c-d5Elo(Ot_GA3) + **EPA** I. c-d6Elo(Tp_GA2) + **GLA** J. c-d6Elo(Pp_GA2) + **SDA**.

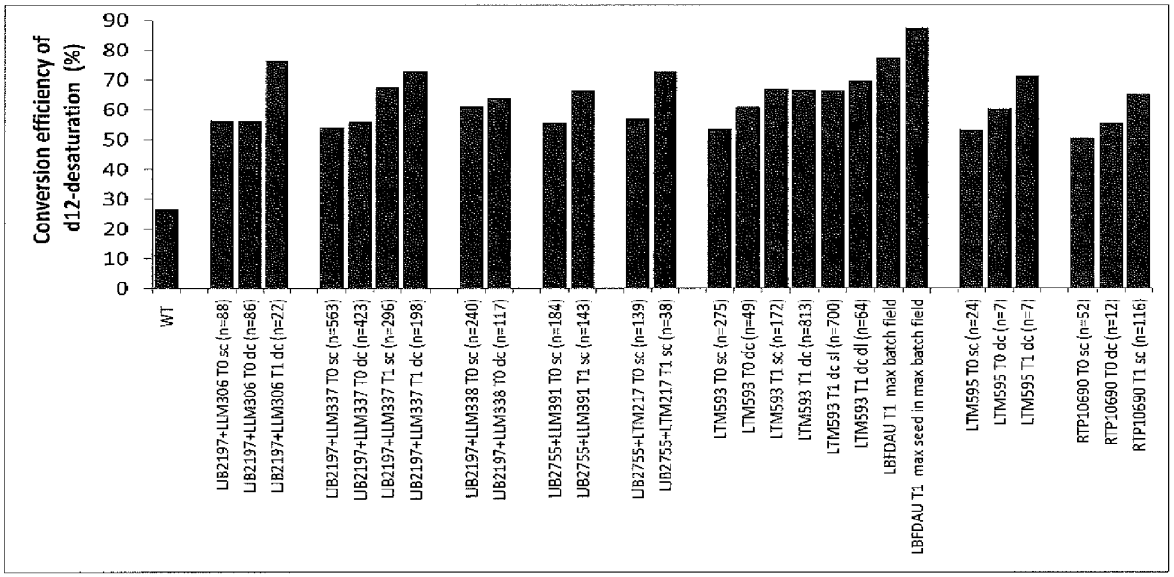


Figure 32: Conversion efficiecies of delta-12-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. Shown are average conversien efficiencies of various plant populations, as well as the conversion efficiencies observed in a seedbacth of event LBFDAU having highest EPA+DHA levels, and those efficiencies observed in a single seed of that seedbatch, where this single seed had highest EPA+DHA levels among all 95 measured single seeds. Data were taken from Example 10 to Example 18. T0 and T1 designates the plant generation producing the seeds (all grown in the greenhouse except for the two LBFDAU datapoints)

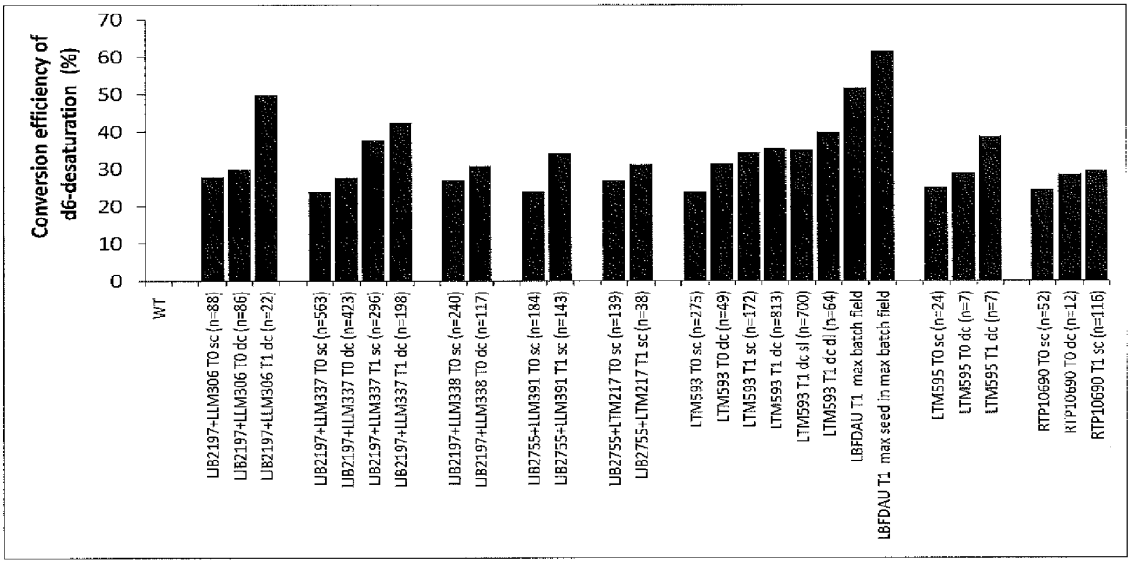


Figure 33: Conversion efficiecies of delta-6-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

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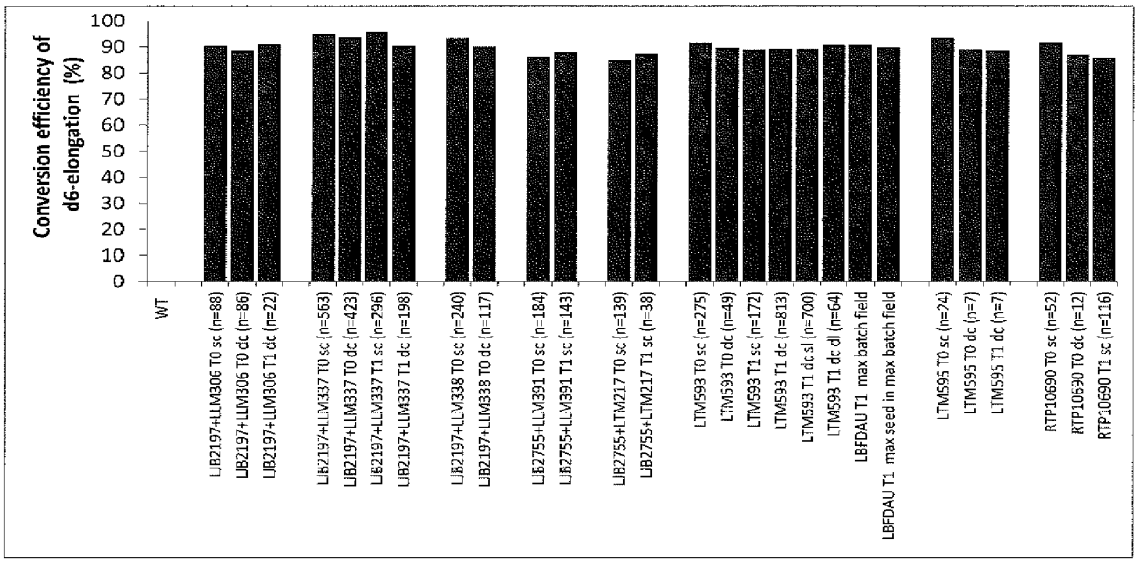

Figure 34: Conversion efficiecies of delta-6-elongation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

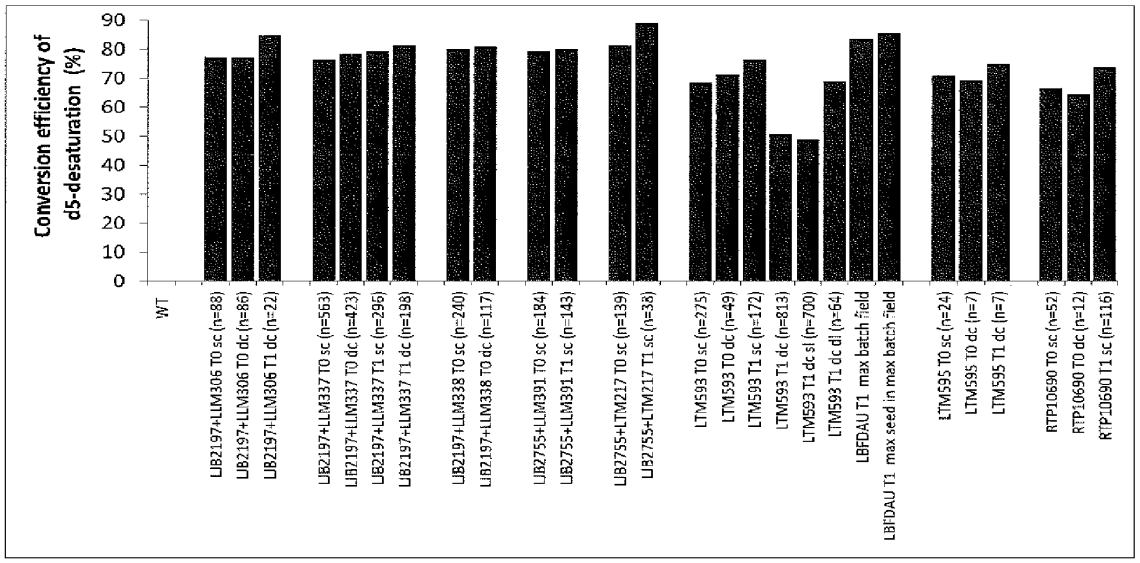

Figure 35: Conversion efficiecies of delta-5-desaturation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

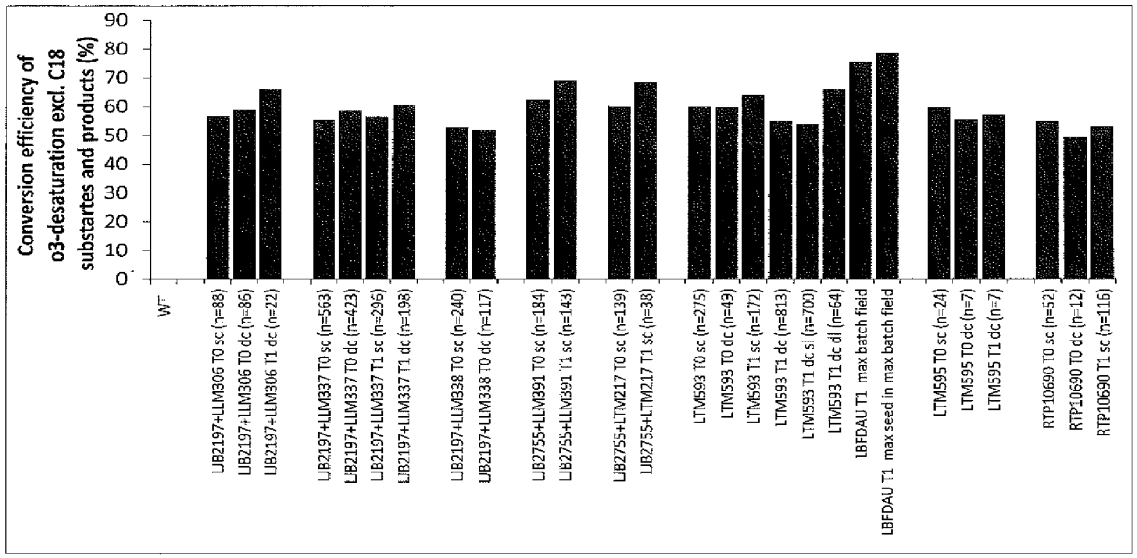

Figure 36: Conversion efficiecies of omega-3 desaturation (excluding C18 fatty acids) in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

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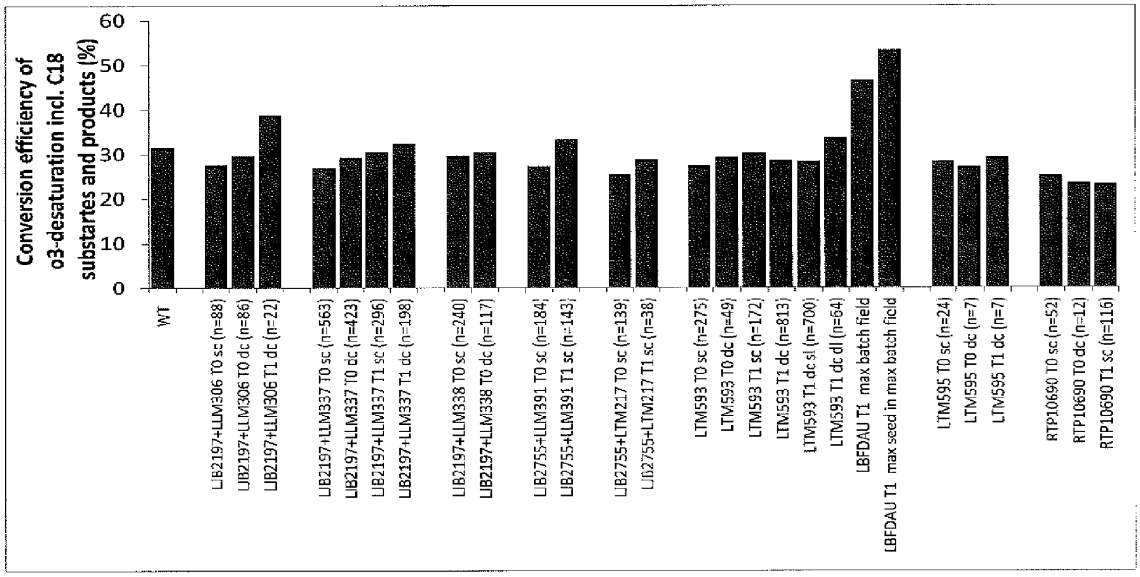

Figure 37: Conversion efficiecies of omega-3 desaturation (including C18 fatty acids) in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

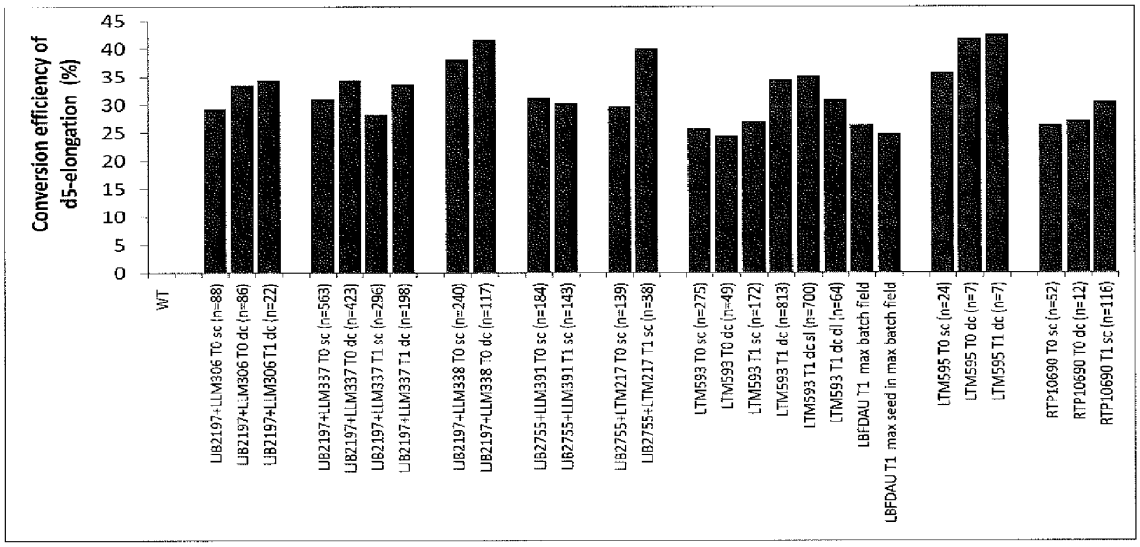

Figure 38: Conversion efficiecies of delta-5-elongation in seed of transgenic Brassica napus and in Brassica napus wildtype seeds. See caption in Figure 32 for further details.

CSIRO Exhibit 1004

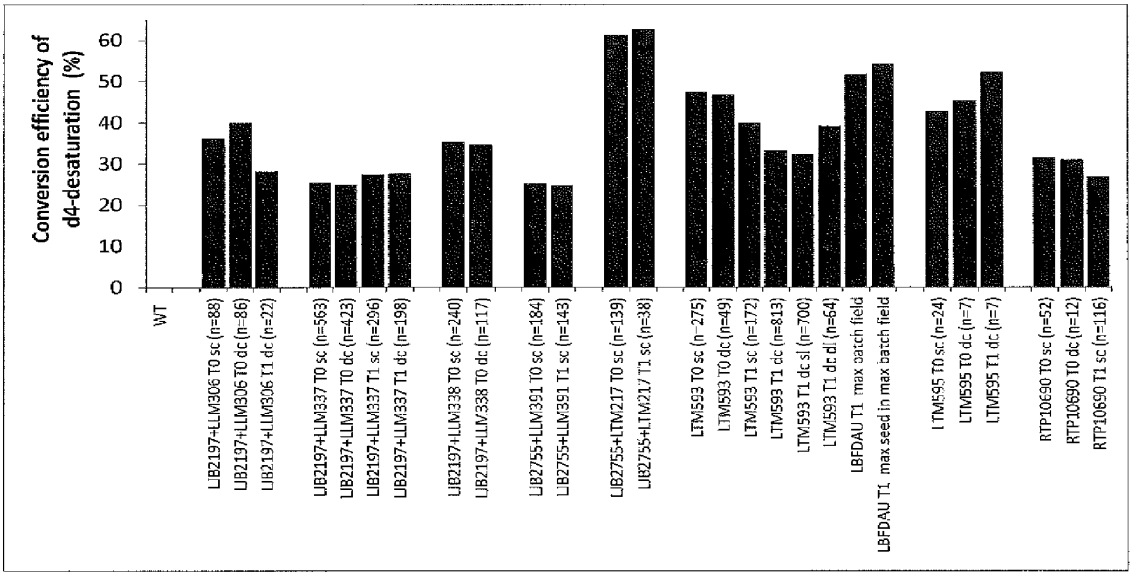

Figure 39: Conversion efficiecies of delta-4-desturation in seed of transgenic *Brassica napus* and in *Brassica napus* wildtype seeds. See caption in Figure 32 for further details.

CSIRO Exhibit 1004

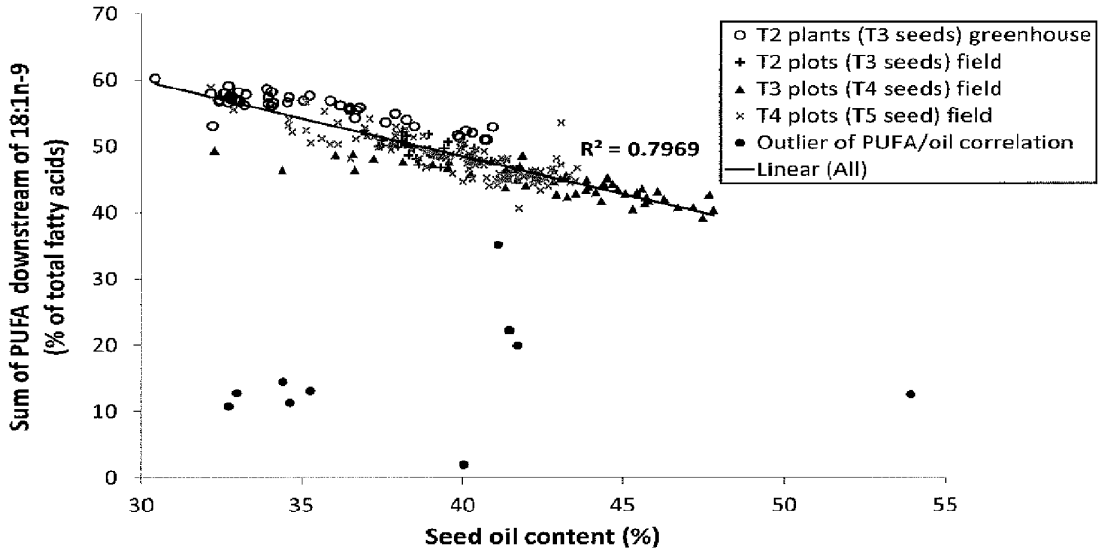

Figure 40: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

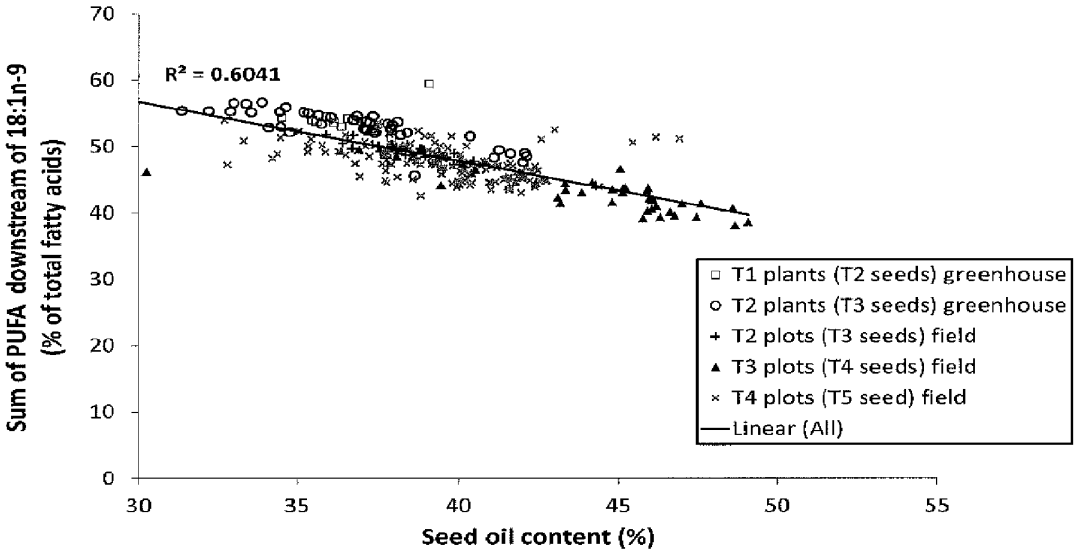

Figure 41: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

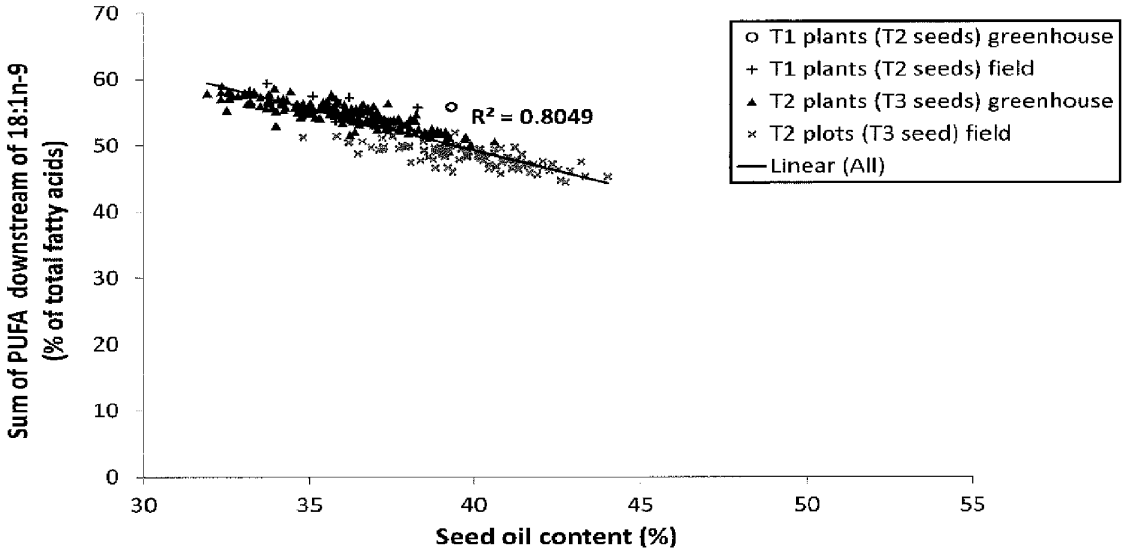

Figure 42: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

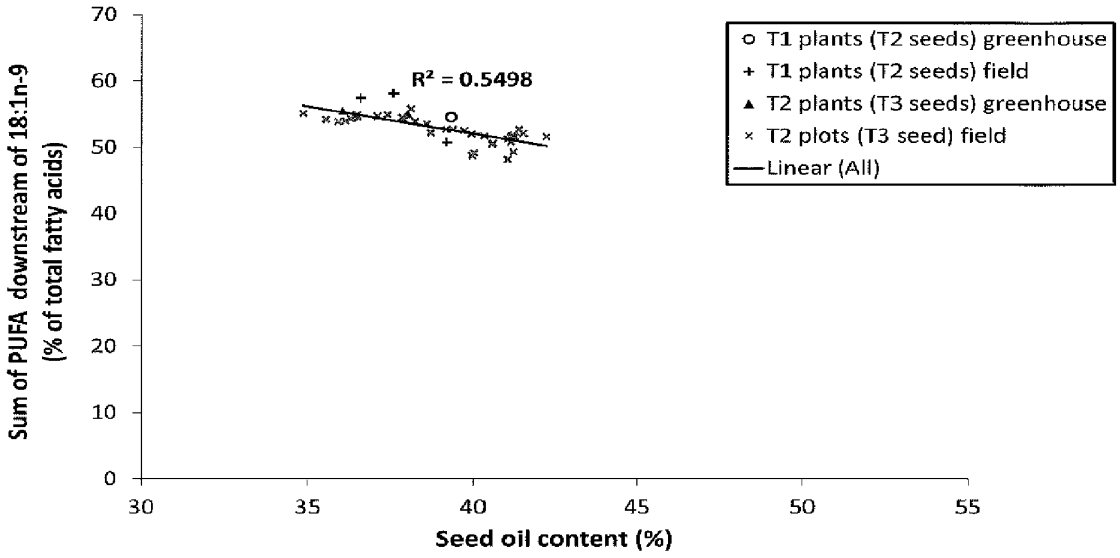

Figure 43: The sum of all pathway fatty acids was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

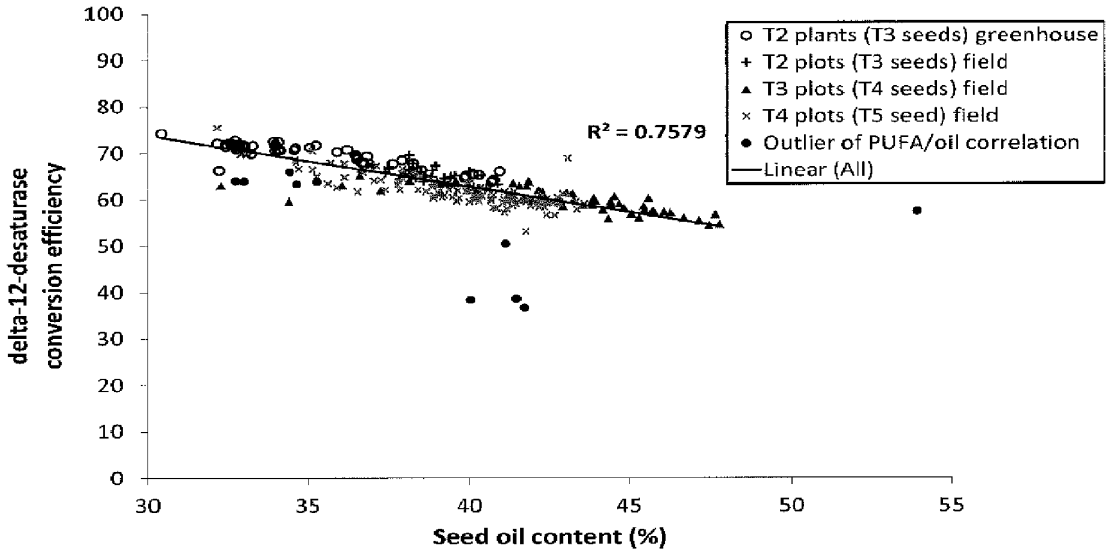

Figure 44: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds to one seed batch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

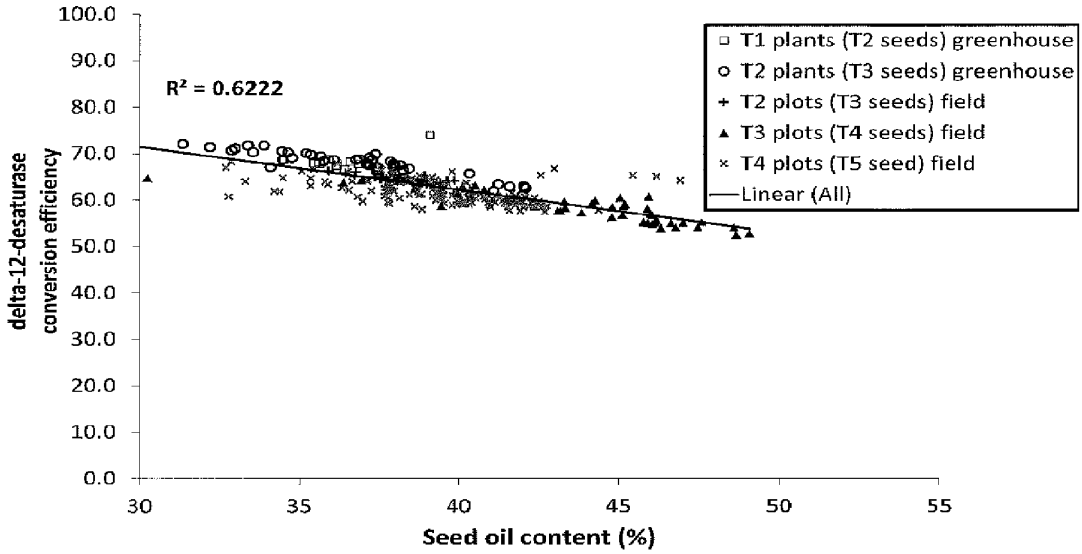

Figure 45: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds to one seed batch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

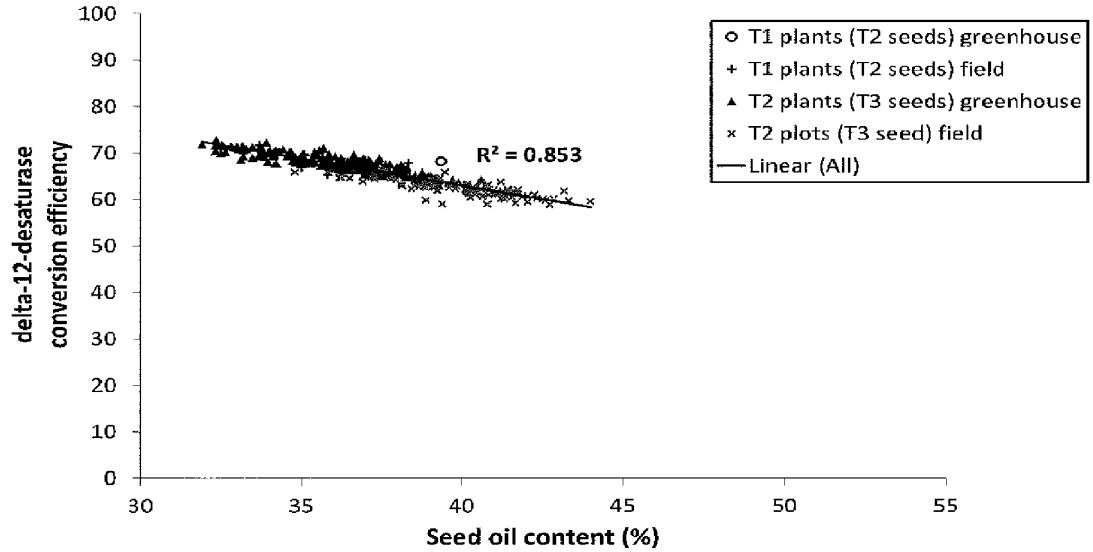

Figure 46: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker

corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

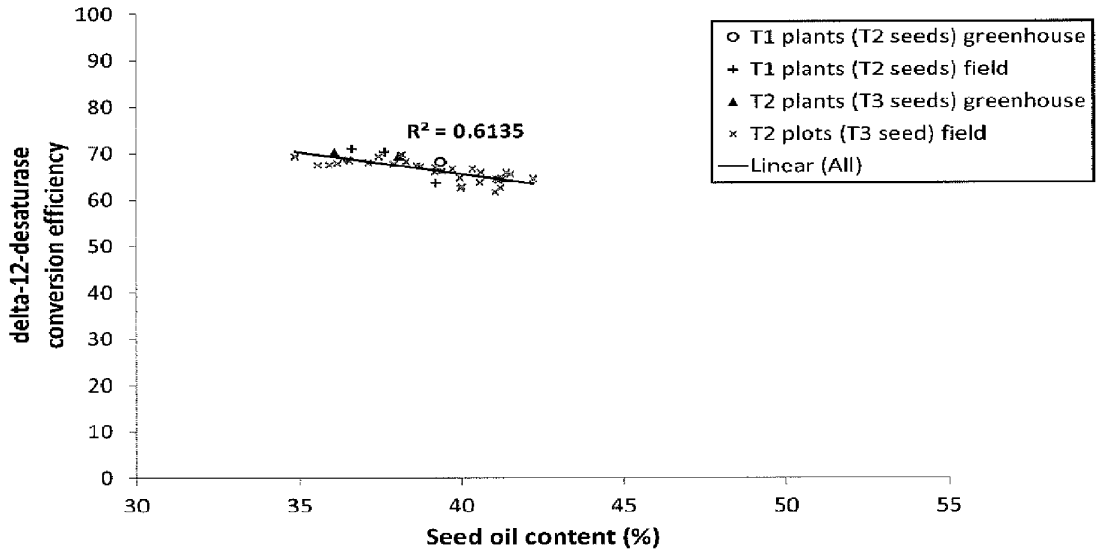

Figure 47: The conversion efficiency of the delta-12-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

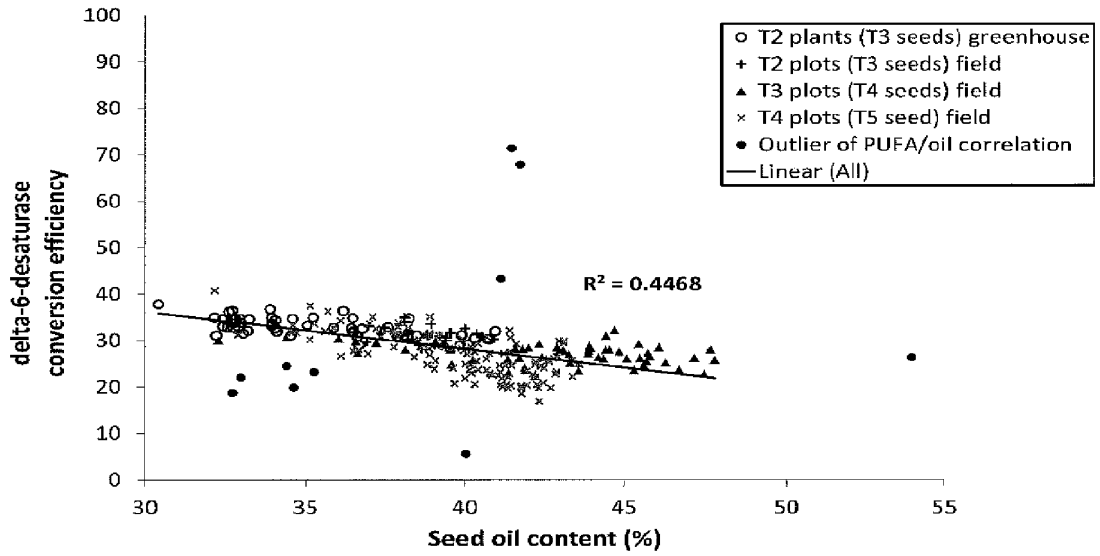

Figure 48: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

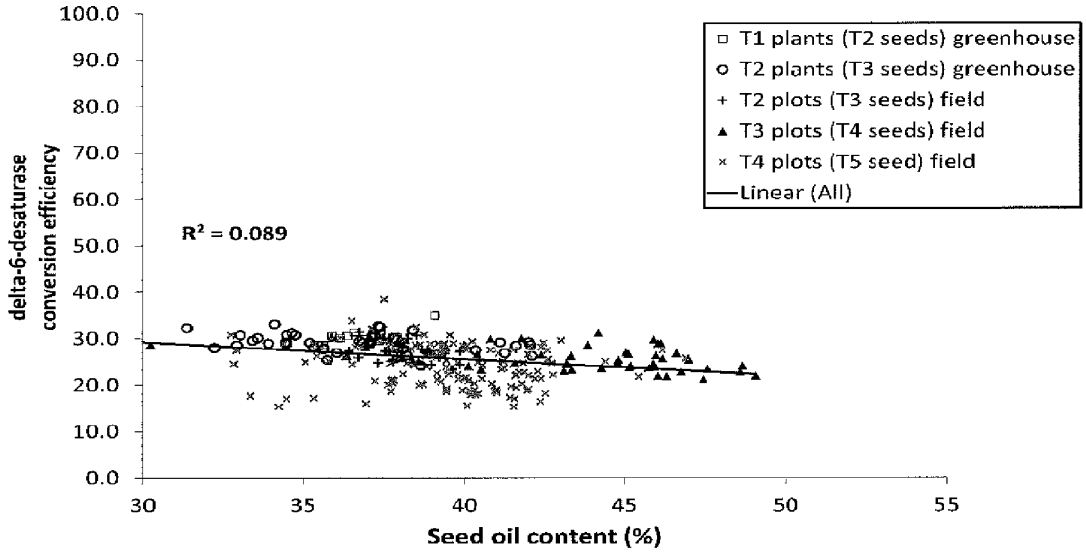

Figure 49: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

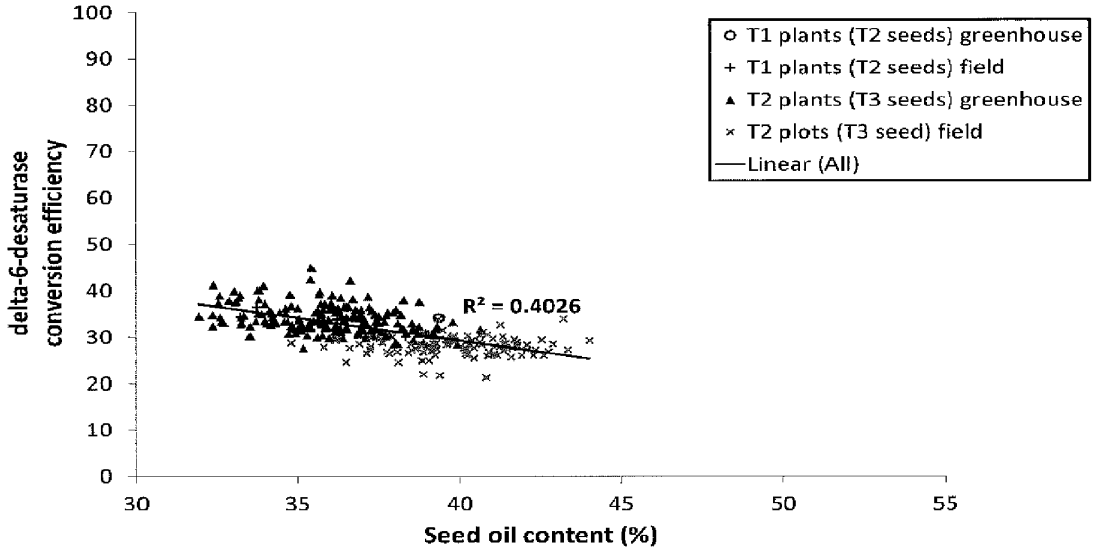

Figure 50: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker

corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

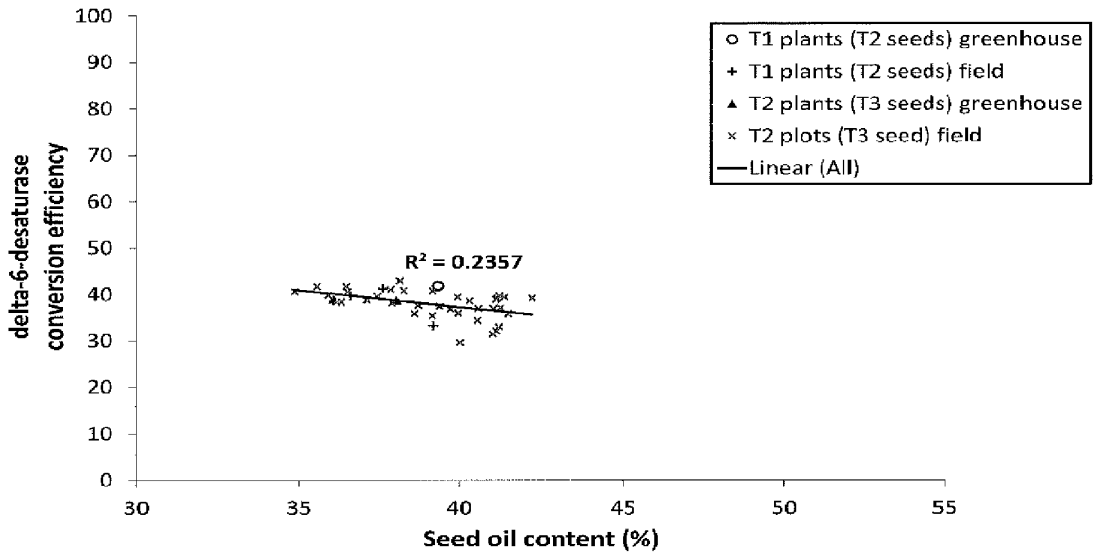

Figure 51: The conversion efficiency of the delta-6-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

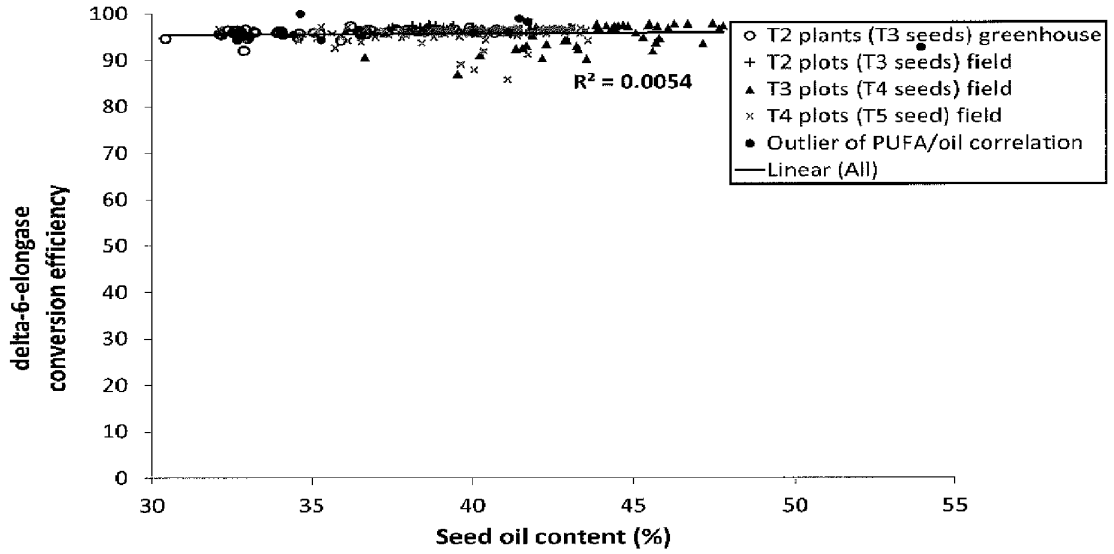

Figure 52: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

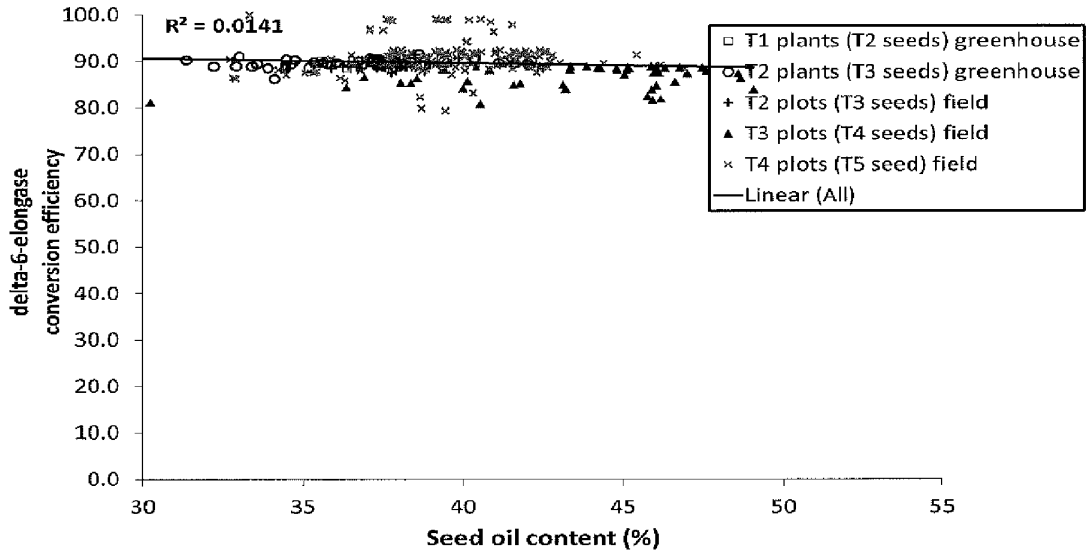

Figure 53: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

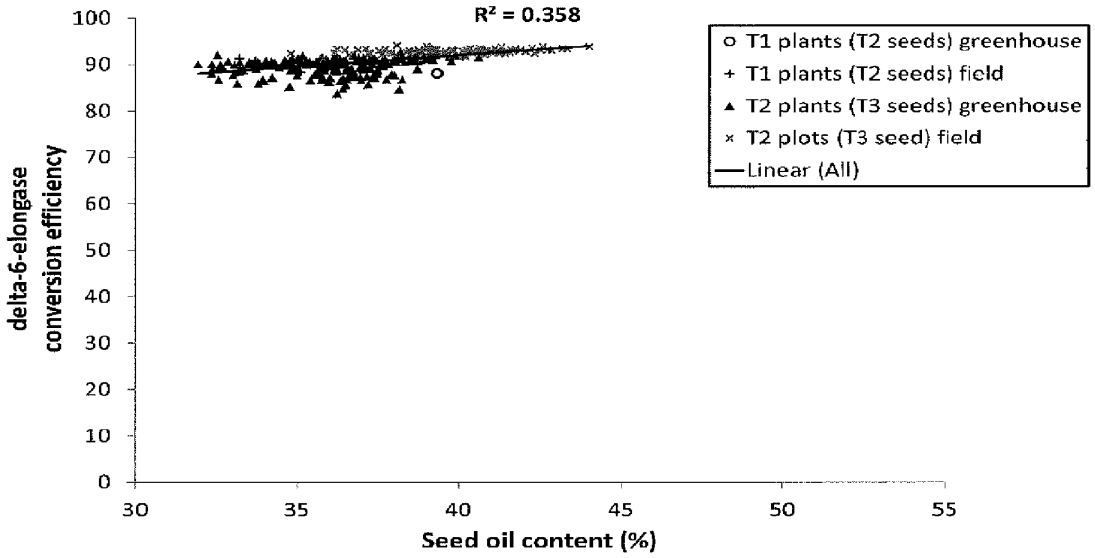

Figure 54: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

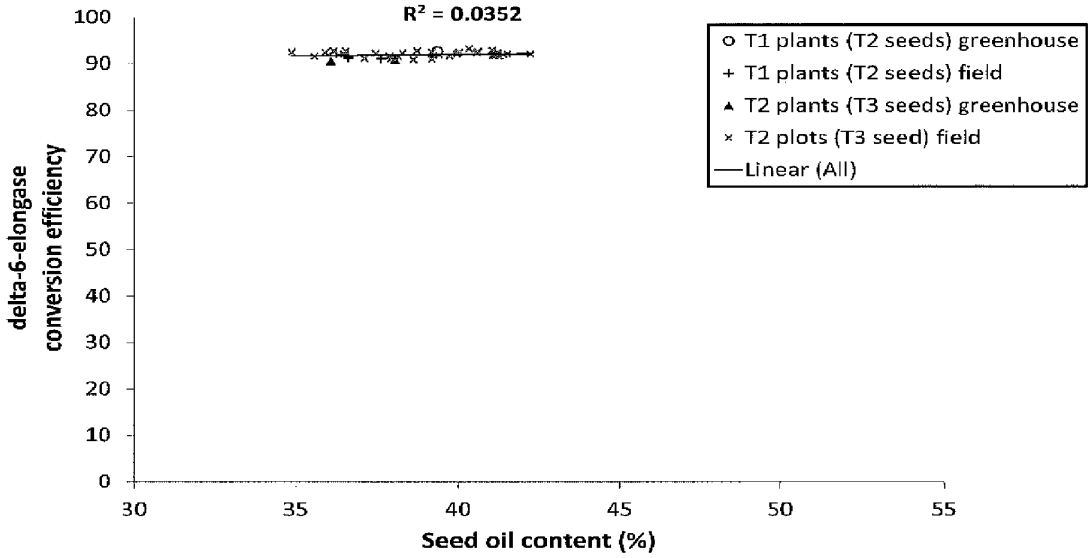

Figure 55: The conversion efficiency of the delta-6 elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

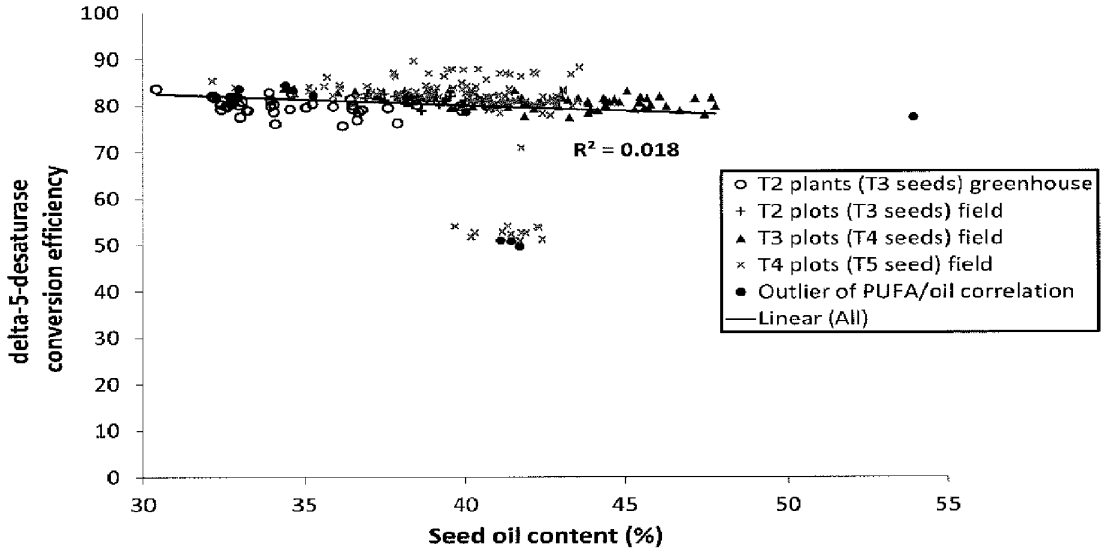

Figure 56: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

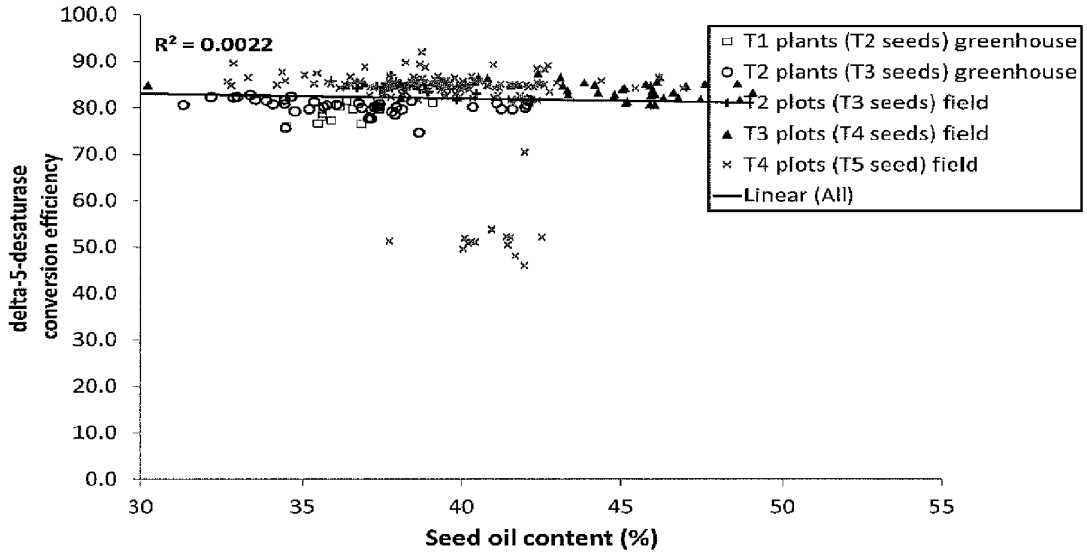

Figure 57: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

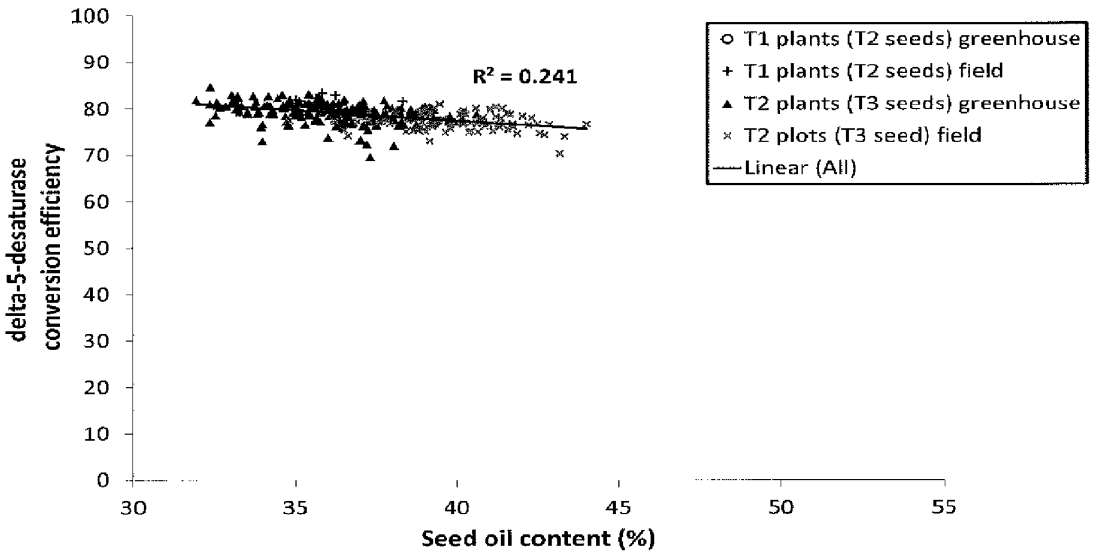

Figure 58: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

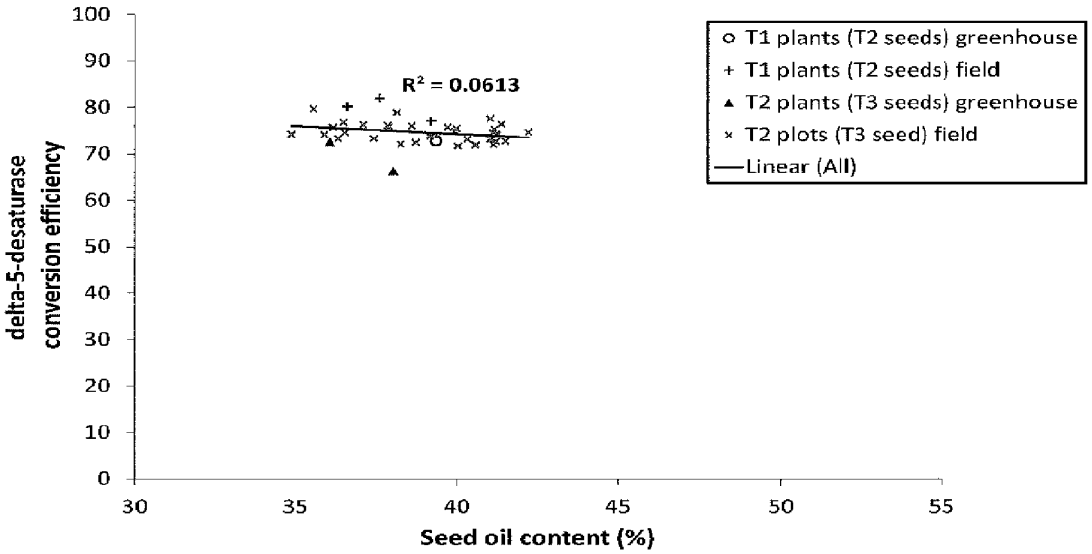

Figure 59: The conversion efficiency of the delta-5-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

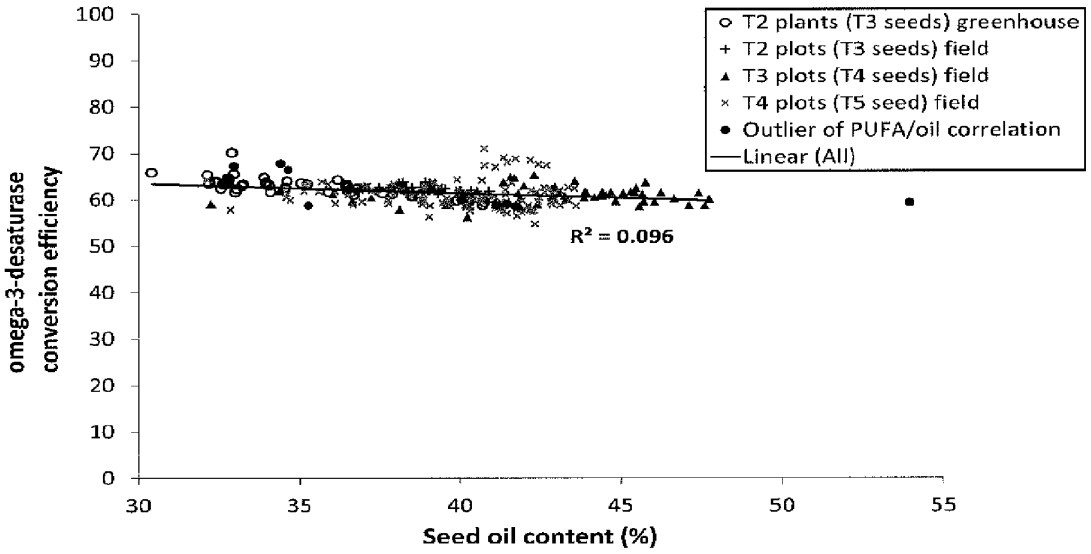

Figure 60: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

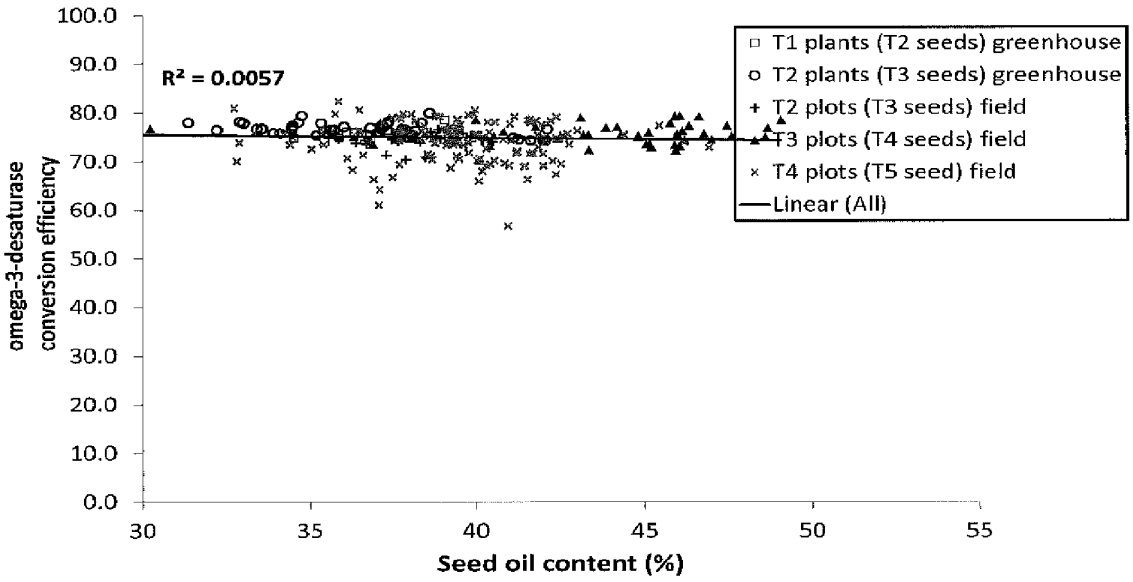

Figure 61: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

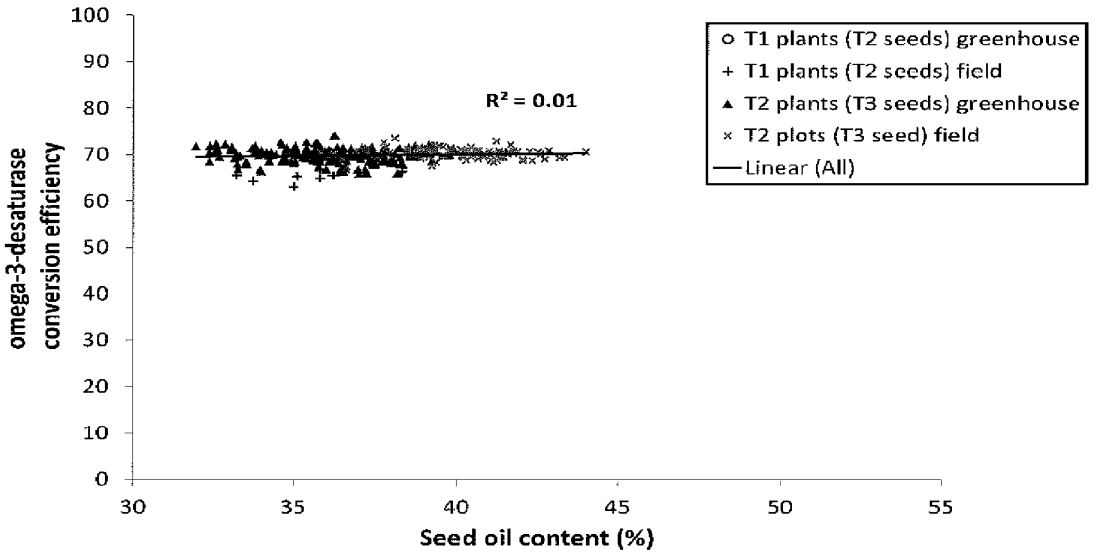

Figure 62: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

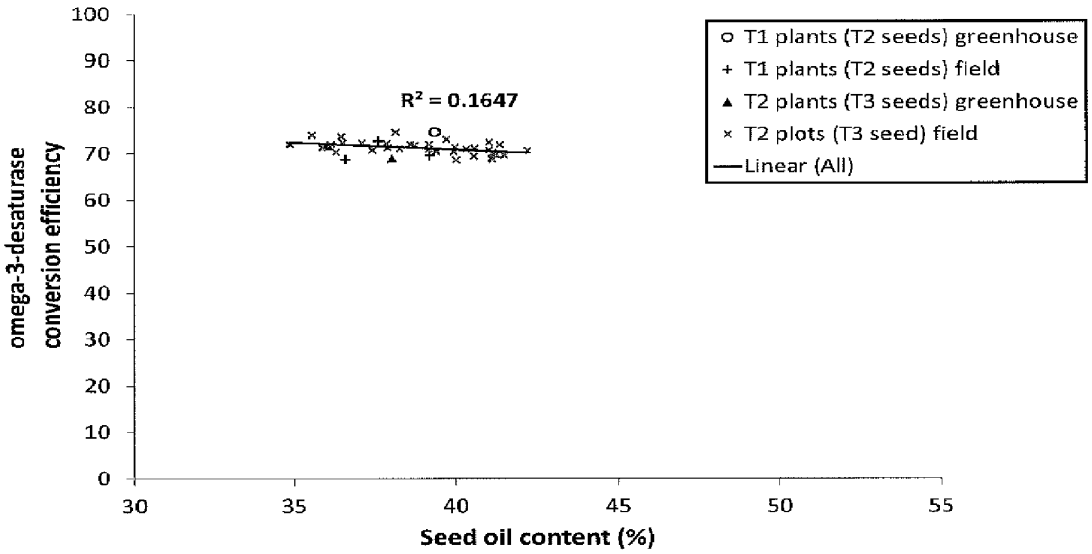

Figure 63: The conversion efficiency of the omega-3-desaturase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

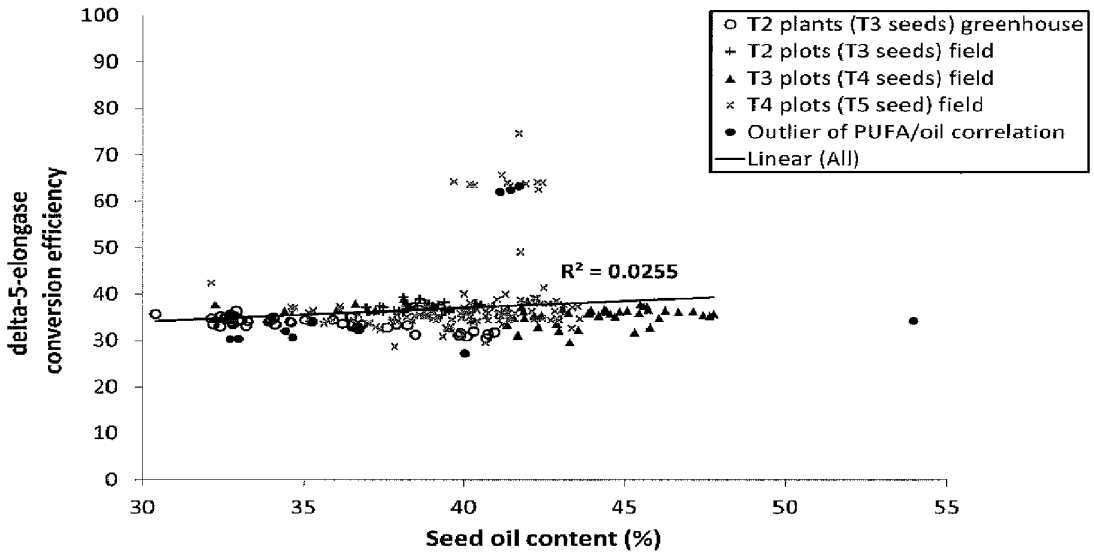

Figure 64: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

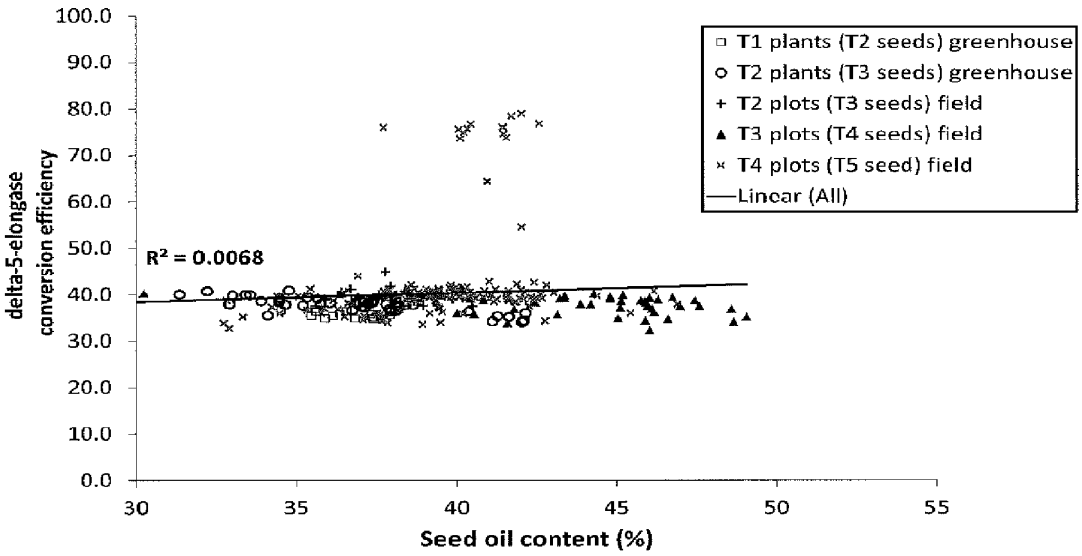

Figure 65: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

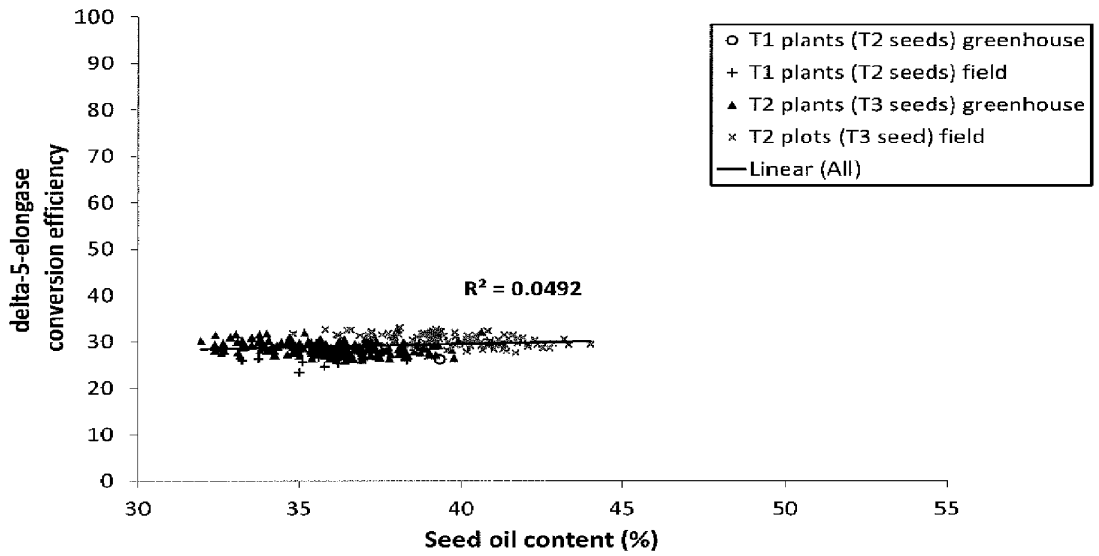

Figure 66: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

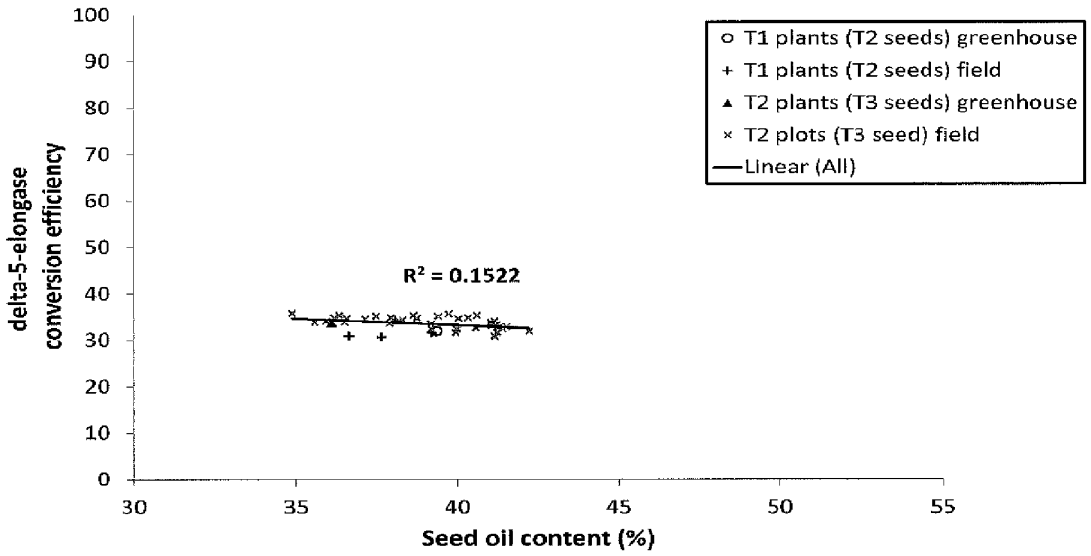

Figure 67: The conversion efficiency of the delta-5-elongase was not correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

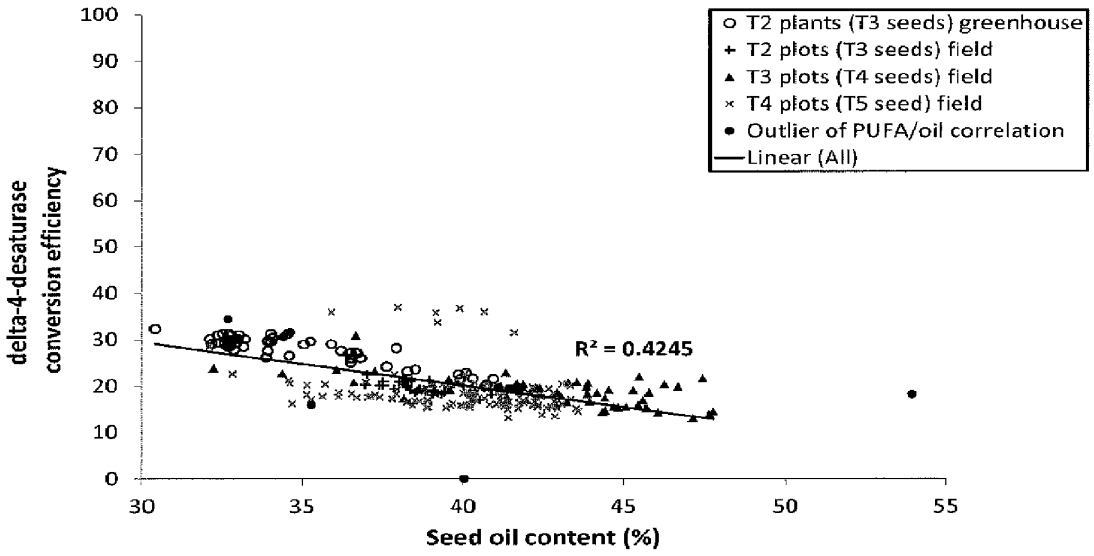

Figure 68: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds to one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

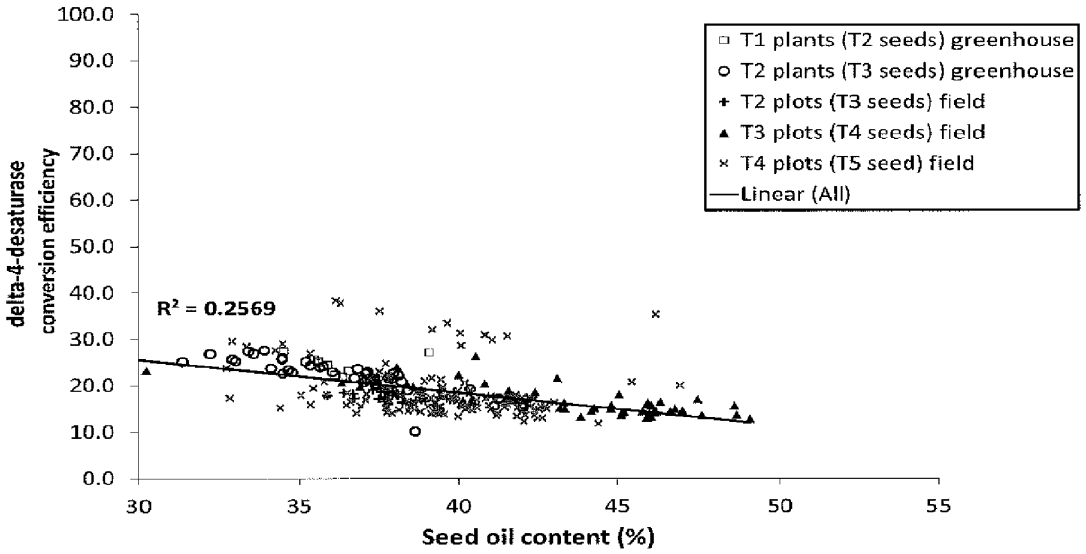

Figure 69: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds to one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

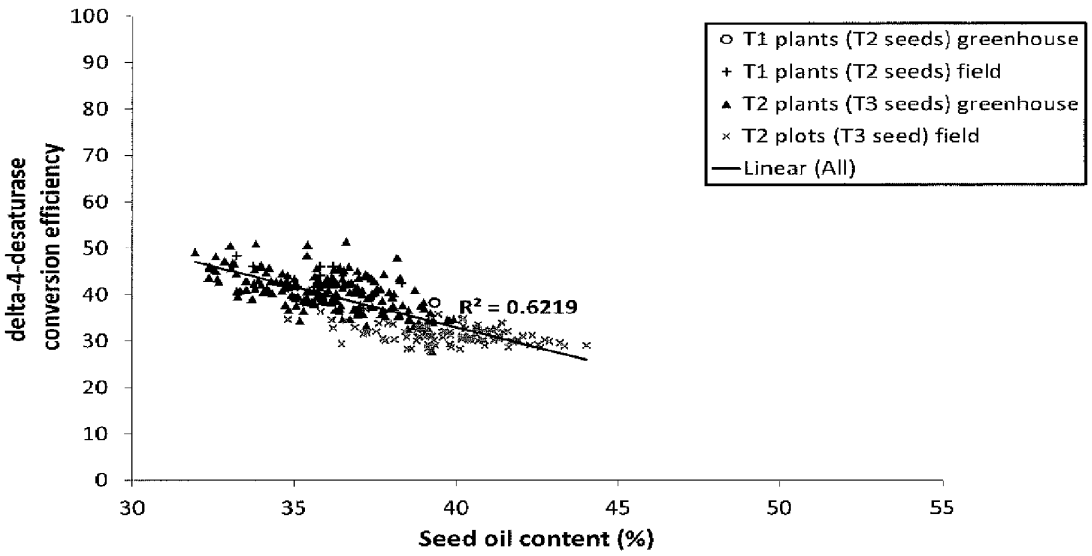

Figure 70: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

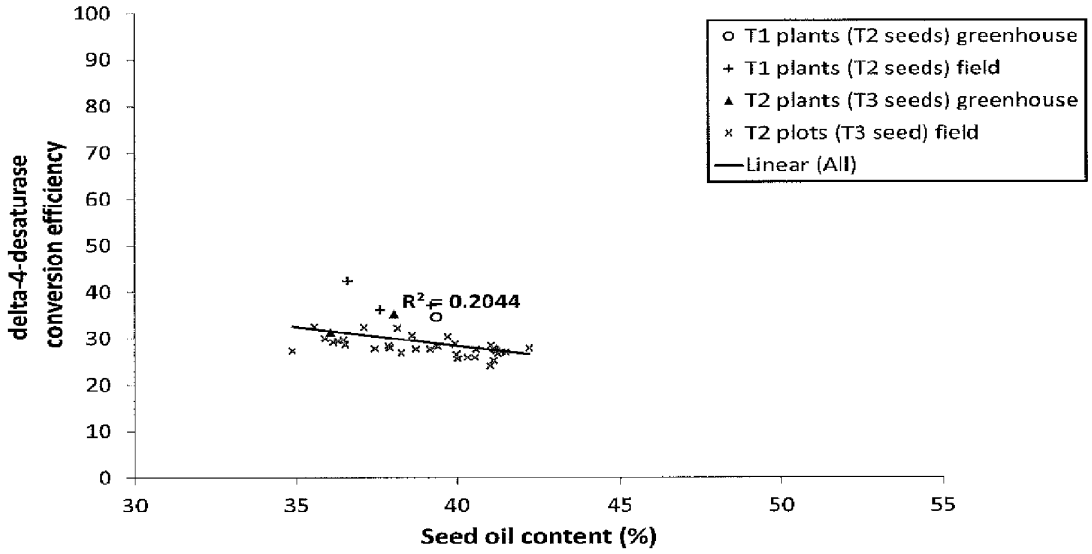

Figure 71: The conversion efficiency of the delta-4-desaturase was negatively correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

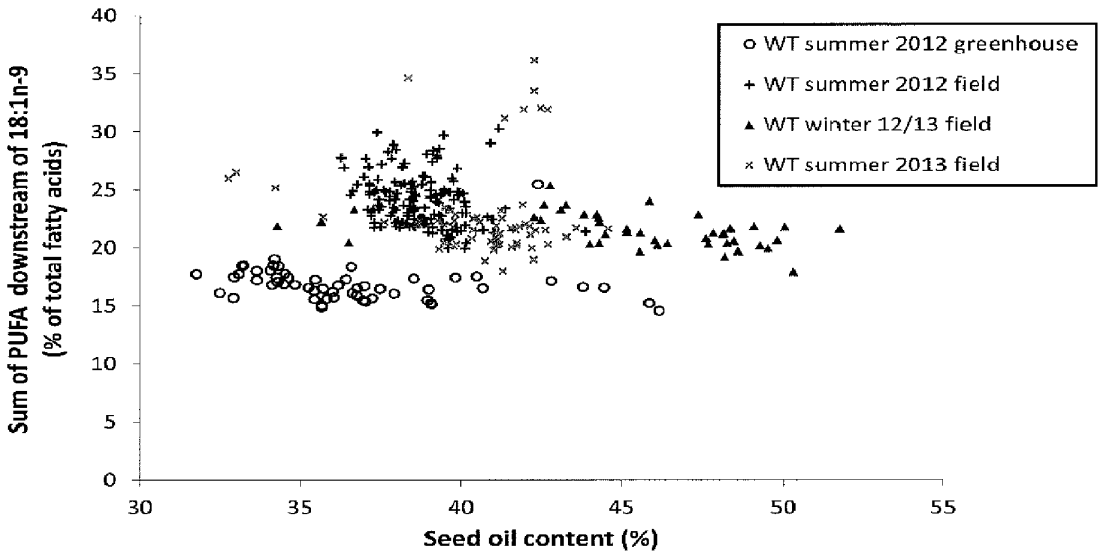

Figure 72: The sum of all pathway fatty acids was not correlated with seed oil content in wildtype canola, but differs between greenhouse and field. Shown are data of three seasons. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

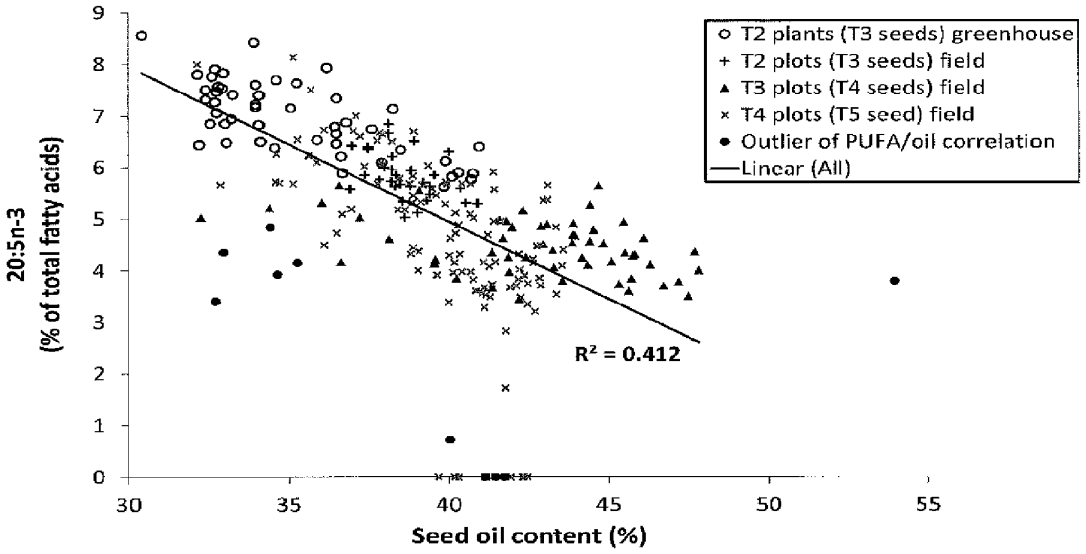

Figure 73: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

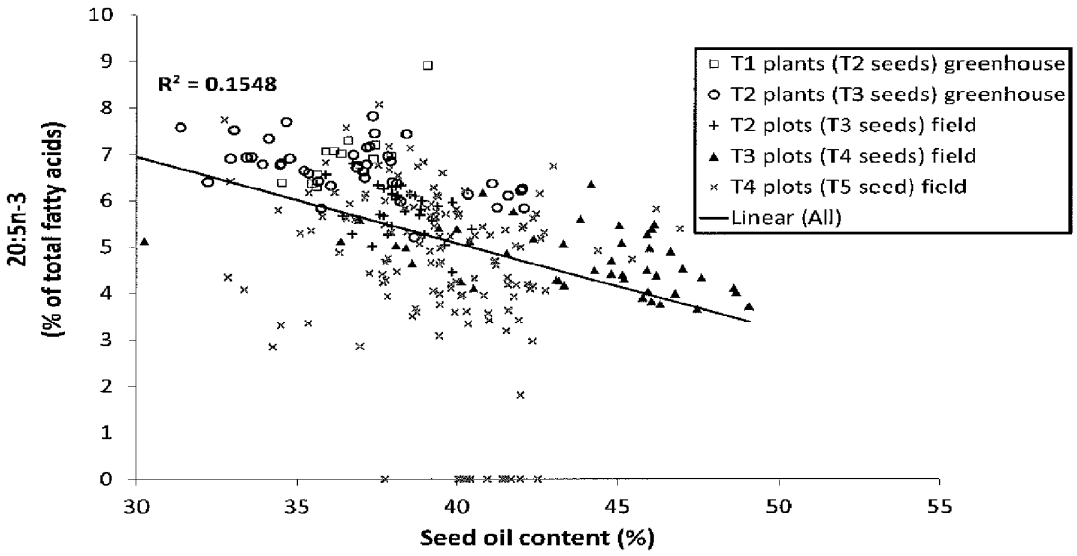

Figure 74: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

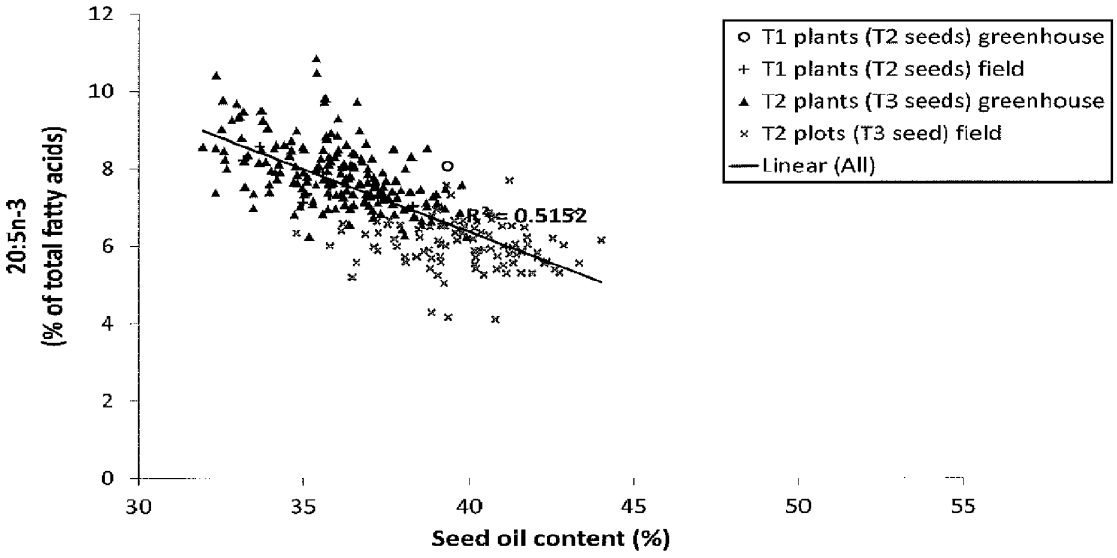

Figure 75: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

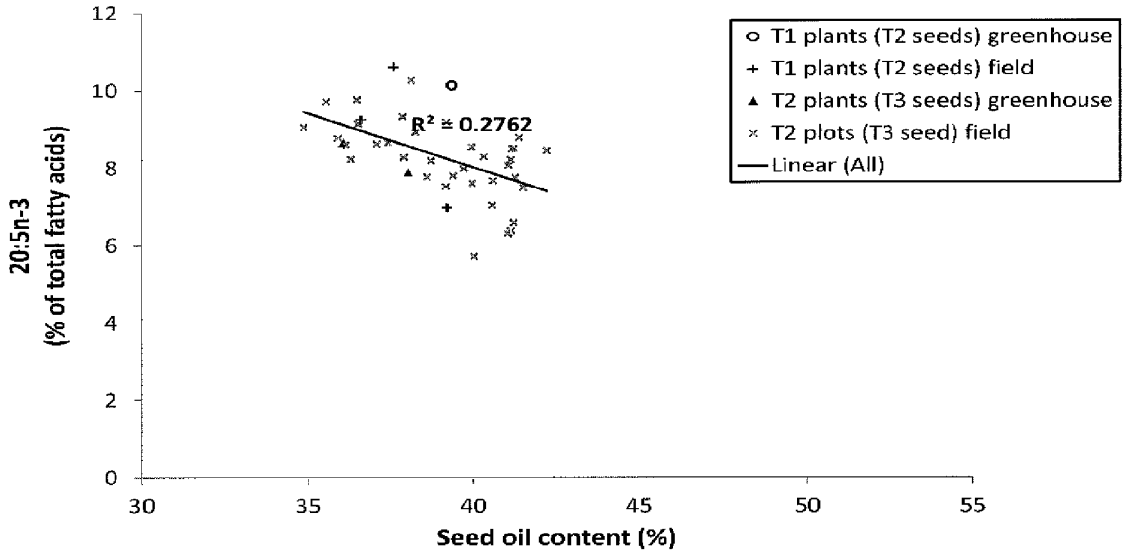

Figure 76: The total fatty acid percentage of 20:5n-3 (EPA) correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

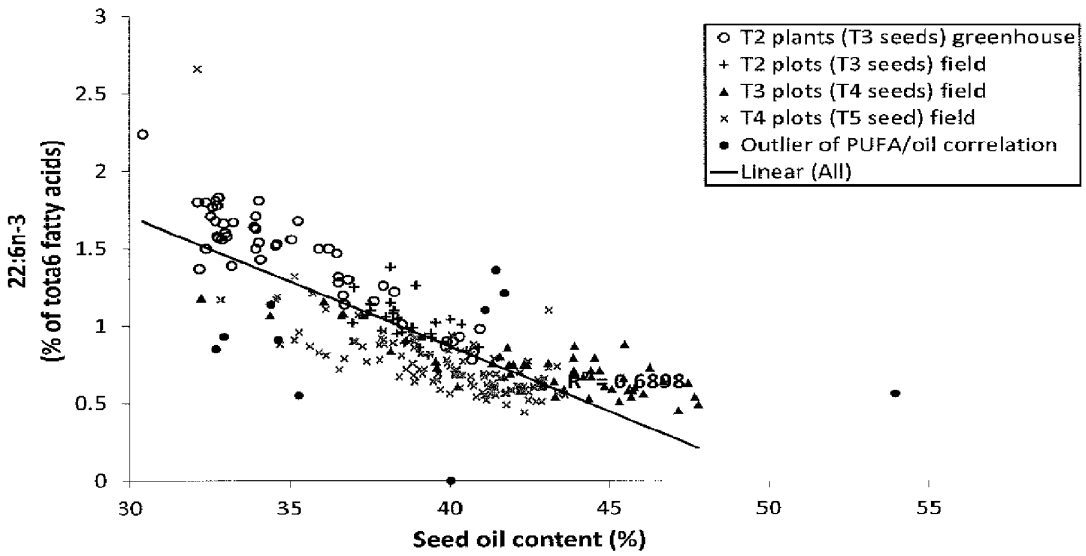

Figure 77: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 3 generations of event LANPMZ. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

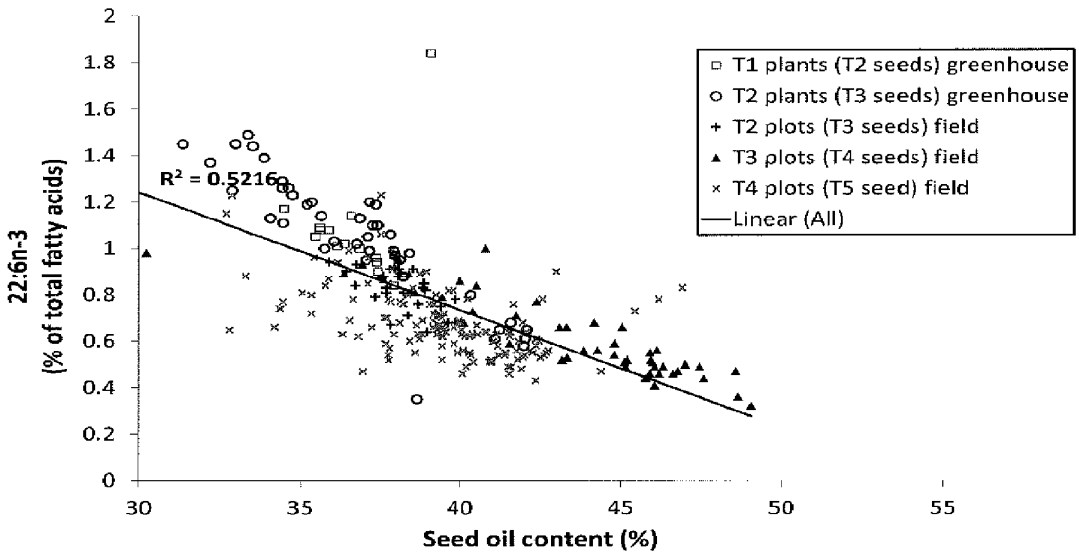

Figure 78: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 4 generations of event LAODDN. For the greenhouse data, one marker corresponds one seedbatch of one plant, for the field data, one marker corresponds to an analysis on a random selection of seeds representing one plot.

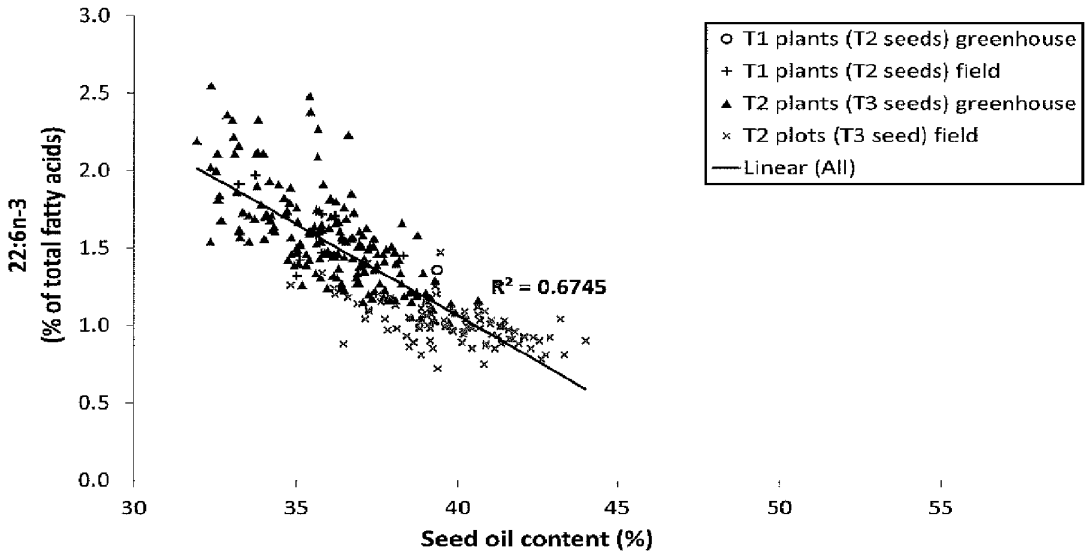

Figure 79: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 2 generations of event LBFGKN. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 50 T2 seedbatches, or 182 T3 seedbatches, for the field data, one marker corresponds to an analysis of one T2 seedbtach of one T1 plant, or the analysis of a random selection of T3 seeds representing plots (36 plots) or single plants (60 plants).

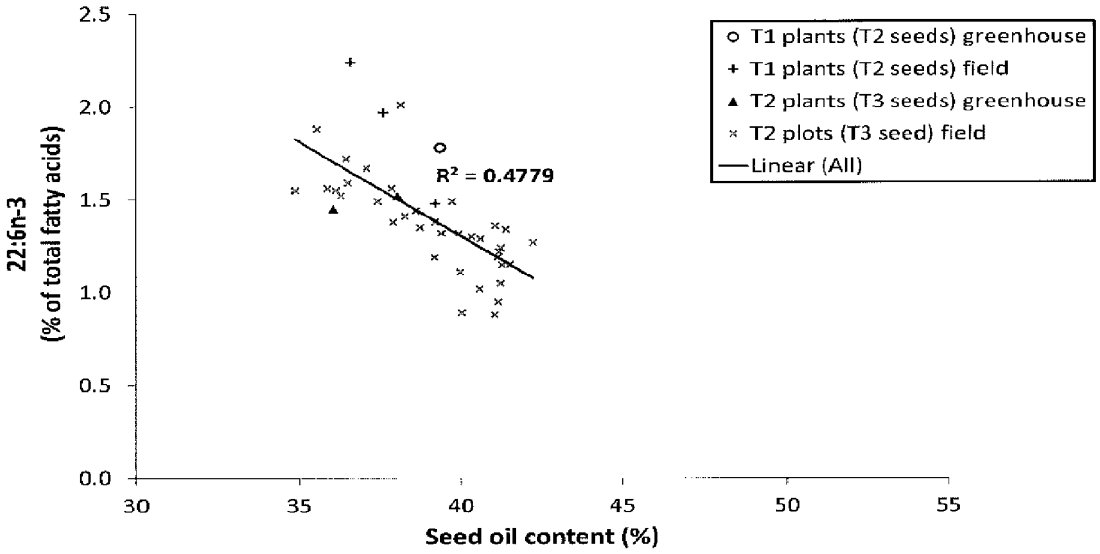

Figure 80: The total fatty acid percentage of 22:6n-3 (DHA) correlated with seed oil content. Shown are data of 2 generations of event LBFLFK. For the greenhouse data, one marker corresponds the analysis of a random selection of seeds representing a bulk of 10 T2 seedbatches, or 195 T3 seedbatches, for the field data, one marker corresponds to an analysis of 1 T2 seedbtach of one T1 plant, or a the analysis of a random selection of T3 seeds representing one plot.

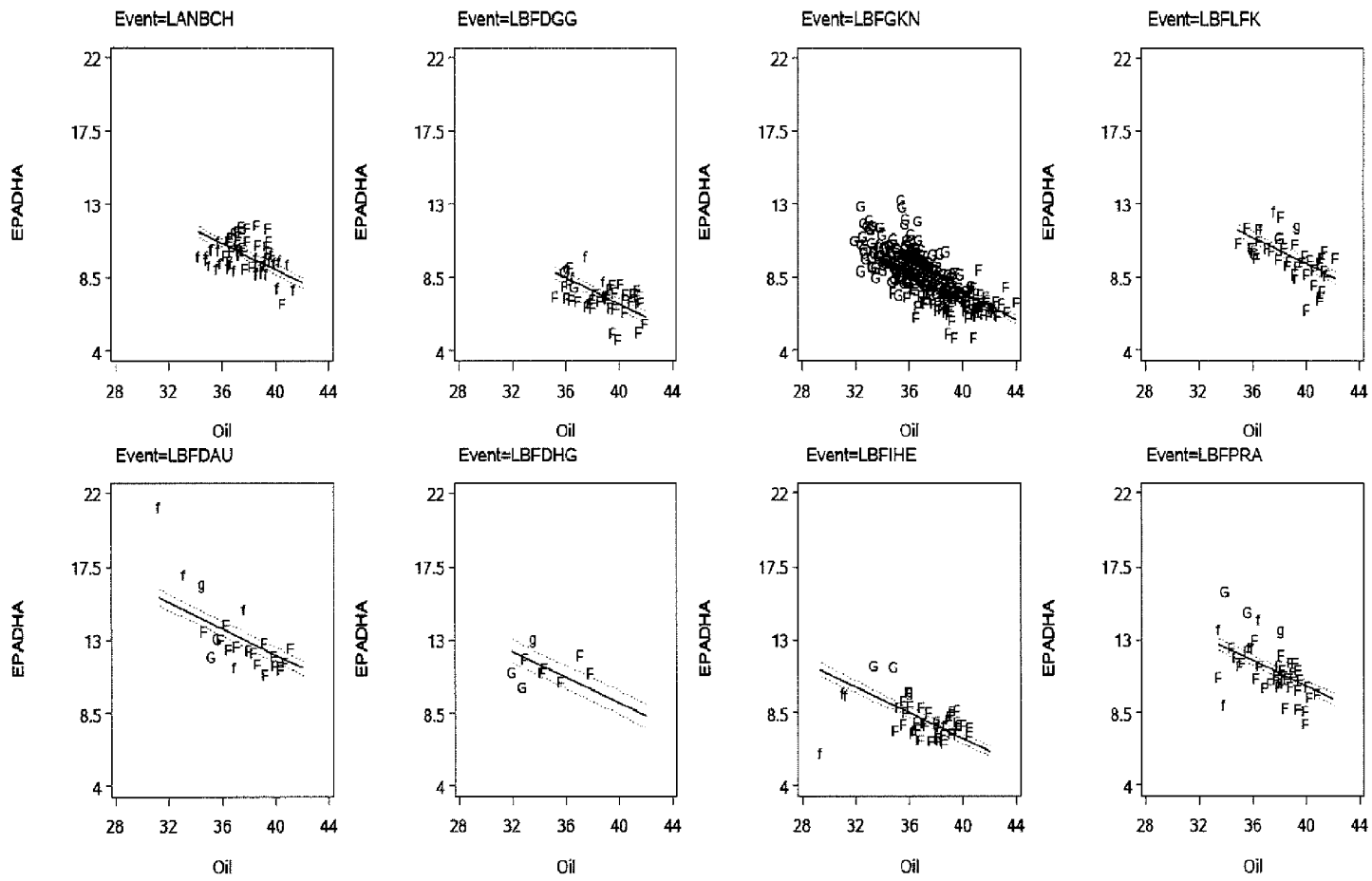

Figure 81: The levels of EPA+DHA (20:5n-3 and 20:6n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

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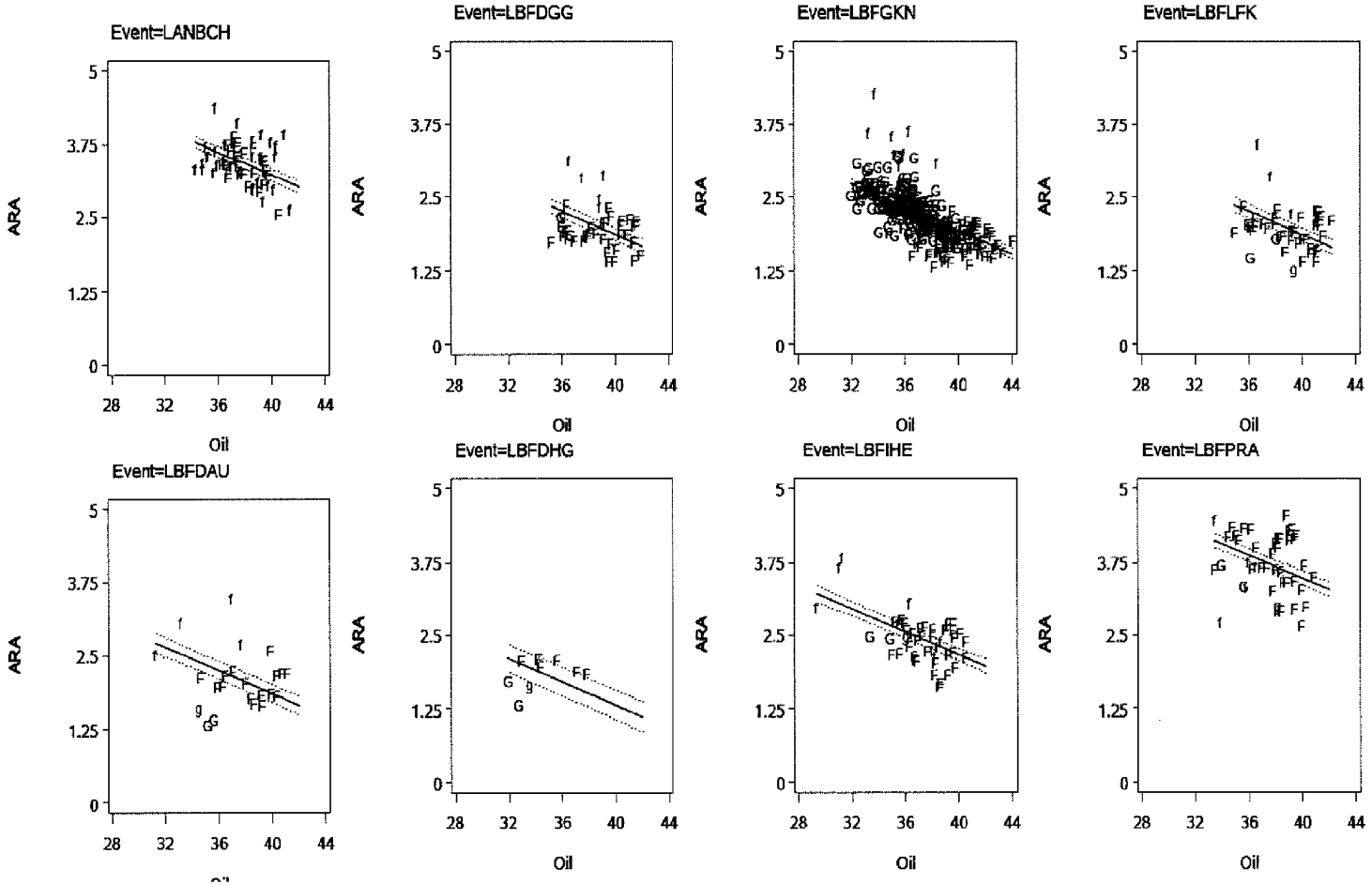

Figure 82: The levels of ARA (20:4n-6) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

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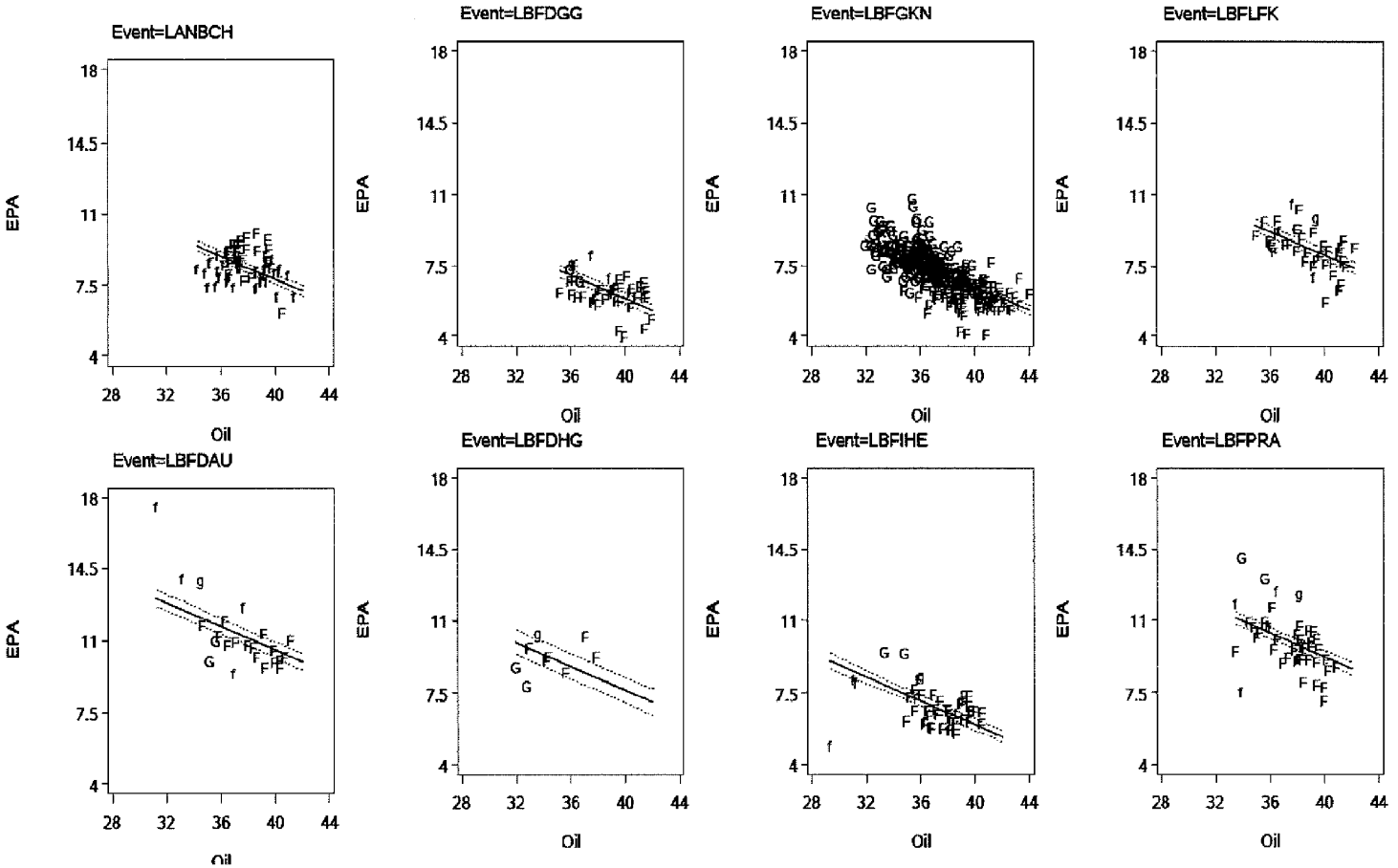

Figure 83: The levels of EPA (20:5n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

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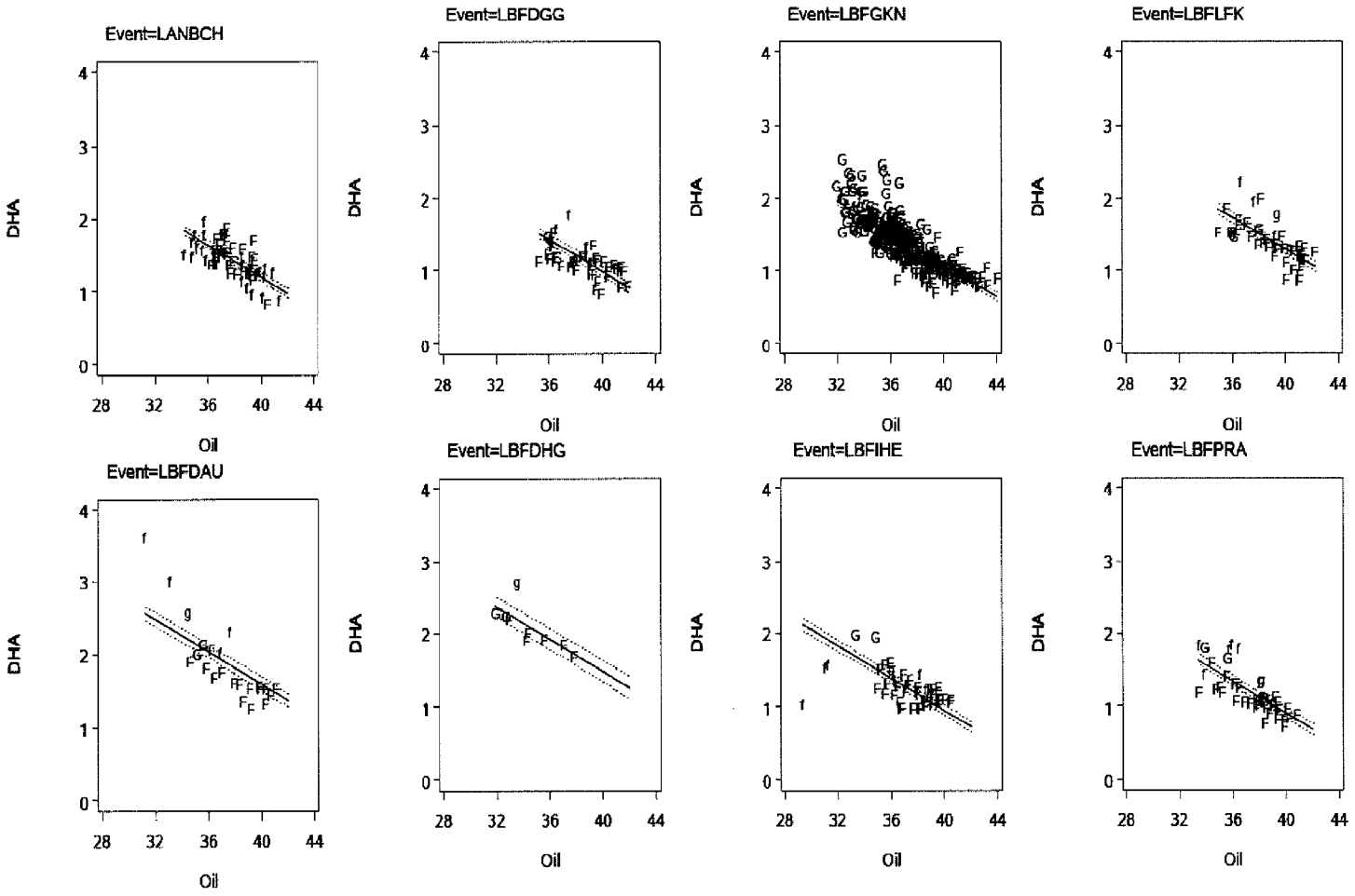

Figure 84: The levels of DHA (22:6n-3) correlated with seed oil content. Shown are data of homozygous plants (single plant: capital G or F, plots: lower case f, grown greenhouses: G, grown in field trials: f and F). The data are described in more detail in Example 12 (event LANBCH), and example 14 (all other events).

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Sequence submission?:	Yes
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Title:	MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS
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Total Drawing Sheets:	80
Small Entity?:	No
Petition Included?:	No
Secrecy Order in Parent Appl?:	No

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Representative Customer Number: 29137

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Foreign Priority Information

Applicant Information
Assignee

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Postal or Zip Code of mailing address:	67056

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Application Number:	62234373
International Application Number:	
Confirmation Number:	4370
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First Named Inventor/Applicant Name:	Toralf Senger
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Application Type:	Provisional

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Deposit Account	503852
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Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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National Stage of an International Application under 35 U.S.C. 371
If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office
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SEQUENCE LISTING

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<120> MATERIALS AND METHODS FOR PUFA PRODUCTION, AND PUFA-CONTAINING COMPOSITIONS

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<220>
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actaaggatg acctacccat tctt 204

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acagatgaat actagctgtt gttcaca 147

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gatcactgat tagtaactat tacgtaagcc tacgtagcgt cacgtgacgt tagctaacgc 240

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tggccactaa ggccaatttc gcgccctgca gcaaatttac aca 343

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<211> 168
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<400> 61
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<400> 62
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<210> 63
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<400> 65
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<210> 73
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gaaagcatac atattattag caaatcttca ttatttaaaa tcattaatta ctatatatca 300

taatcacatt aggtaattct gtaggttttt atttaagaaa ataatatata ataaatagca 360

accttgctat atcatatgat atcatatagt tggtattaaa tgtttctagt gacatataaa 420

aatcgaattg gacgaacatg tttttcaatt tcagtgtggg actgacacat atgactaatt 480

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caatcaattt gtttacactc aaacactgat agtttaaact gaaggcgggg attggggagg 660

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cactgattag taactaaggc ctttaattaa tctagaggcg cgccgggccc cctgcaggga 780

gctaagccgg ccaatttaaa ttgatatcgg tacatcgatt acgccaagct atcaactttg 840

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agcaaattta cacattgcca ctaaacgtct aaacccttgt aatttgtttt tgttttacta 960

tgtgtgttat gtatttgatt tgcgataaat ttttatattt ggtactaaat ttataacacc 1020

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<210> 213
<211> 1226
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 213
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ttaactagta aactaaagta gtcaaaaatt gtactttagt ttaaaatatt acatgaataa 180

tccaaaacga catttatgtg aaacaaaaac aatatgtcga ggcgatcgca gtacttaatc 240

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ttcaggtgta tgctcacaag gtctataaat atcttgagac tccactgctg tctctgagtg 420

agataagaga caacgaccgt attgtcgcat ataggttcaa ccagatgcac agaggaccag 480

gaaaagccaa actagagatt cttcactcgg agcagaaaca gtaagcattc gagaattctt 540

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tggaaagtta tttggaattc cccttgttac ttatgtcaac tcagaacgac atagtggaag 660

tgacattgat gcaattattt ctggattgct atcaccgcta caccggactc attcatcatc 720

tggaagctta gaggatacta cagttggtga aaaagagttt tctttcagcg tcttctggtt 780

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gccagagatt cttgtgttcc acctaaaacg gttcgcctat accagatttt taaagagtaa 1140

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taaagagggt gagtcgtatc tttacg 1226

<210> 214
<211> 2100
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 214
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ggttatcatc gcagatggct ccctcttctc atcgcagatg gctcaggtct tccaatcaca 120

catattggtt cctccattga tcacaaaaat tttaaaggaa tttttaacat tttttgtaat 180

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cggctgggta acttggaccc atatttgtgc gtatttatac actccaagcc caaggtggtt 960

cgtcctccac accctgcaac attggctcaa accggtccat tcaagcttgt atccggctcc 1020

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ggtatttgta tttggagggt gttgggatgt cgctaattct tcaaactggt tagagttata 1260

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gcctaaccca gaaaatagaa acgattggtt tttatttgag gtaataatgt gccgtggtat 1500

cggcgggaga atactgtggc gttggccaga tgagattctt tagaaagaag tcaaaggttt 1560

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aacatatcat aagctaagca aatcacacac ctccccaatc catggatatg ttctctcttt 1980

tctcaatcaa tttgtttaca ctcaaacact gatagtttaa actgaaggcg ggaaacgaca 2040

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<210> 215
<211> 2065
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 215
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gccgctagtc tgtgcgcact tgtatcctgc aggtcaatcg tttaaacact gtacggaccg 180

tggcctaata ggccggtacc caactttatt atacatagtt gataattcac tggccggatg 240

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gatttttaca caattataac tttgtttaaa ttaaaatttt attataatat ttttaggtaa 420

ataaaaagtg tggttctaat ggtaaatttc tctgtctgct ccatctatga catcttttgc 480

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ttgtgttgca gttgtttatg atagatttgg ttgtgtccta ctattgtttg cgttcggatt 720

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taaccgcaag attgaatgat gggtaggtaa attttttttt ttttttttgt ttatggtcat 960

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ttcttataat ttaatataaa aatgtaaaac tatacatttt taaatagatg tttaatgtag 1560

tatttccatt tcttatattg tgtatttact tattttaatt ttcttccttt tatatcaaat 1620

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acgggcgggt taattacact aaactatttc atattagaat atattttaat tttataaaat 1860

atttgaatat ttactttata ttaaaataat aaatacaatc tatttttatt tctttttatt 1920

ctaagatcat atcataactt ttattaaaat tttttggttt taattataaa gatcaataac 1980

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<210> 216
<211> 1500
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 216
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tgtttttttta tagatgtggt aattttatat atatctcaag gtctcttgtg gattagatag 180

agagtgaatg attaaatgtt tttaaagtgg gctctcctat tgcaataatc taaaaataaa 240

ggtttgtttg ttatacacaa taatttttaa gagagaattt taaacaaaaa atattactcc 300

aatcaaaaat agcattttct aaaaaatatc tcattctcaa ttgatataat ttcttctttt 360

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gaaaatgctt ttatgcttct aacatttatt tcaaagactt ctgatttatg tttggaacaa 480

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<210> 217
<211> 1948
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 217
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taatcagtga tcagattgtc gtttcccgcc ttcagtttaa actatcagtg ttgctttagt 120

ggtcaatcaa aagatgaaca gtatcttctc gatcgatgat ttctccgatc ccttttggga 180

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tatgcttctc ggtagatcaa atggacataa caacaacaac aatagaatgc cattaactgg 1860

taatagcagg atgggtggtt cttcatgcat cccacctttt caaccgcaat caaatccaaa 1920

catgggggga ctaacaacaa ccattcta 1948

<210> 218
<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 218
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ccatgtctcg aaaaatcttt aaggcggtag tcatatcctg ttgcttcacg tacccatcga 180

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tacacaaccc actcatcaac atattgtaga tggcagcatc cggtgaaact cctctctcta 540

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ctttgttggg atctttcgga gttgagttga ctgatacccc 1600

<210> 219
<211> 955
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 219
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taggattcac atgatccaaa aaggaaatcc agaagcacta atcagacatt ggaagtagga 240

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gaaaaaaaat tggtaattac tctttctttt tctccatatt gaccatcata ctcattgctg 360

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ggggagagca gtccaagtgt gtttttaatc tcagataggc ctaacatcta gatttatttc 480

agactaaccc aaaaccgaaa cttttgcatt caagtcatct ttcgtgttga ttcgtaattc 540

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gagttgtttt ttttttttga acttttgtat ctgatcagtt cggatagact cgaaaaagga 780

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CSIRO Exhibit 1004

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agagaaccca aaattttcac atcatctcaa ttatttttcg taaaaaatac aatac 955

<210> 220
<211> 2199
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 220
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tagtgaagaa gacaaatcaa aatattaatc ttctgctaaa aaactgaaac tttcaagttc 180

caaatttcca aaaaacacat ttctccttaa tagacctttt aacatcaatt caaactacag 240

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gaagaagacg catcaaaaac tgtagcagct aaaaaaaaaa ctgaaacttt caagttccaa 360

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cagttgtagt agaaggaaga gcgagatgat gagagaggca aagaaactgc tgtgagagaa 660

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<210> 221
<211> 1748
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 221
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agagaagcca catgactaat accttgttct ccaacttttt ggttaaaata aatttatcta 600

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gtaaccataa ttaattatgg tttgaatcgg tttaatttac cttaatcaga tccagtttaa 840

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acaatcaaac caaaataaat agaaaactaa aaacccaaca ttgttaacac agttaatagt 1020

gactttagtt ttagaattta gtatattgta tattgctatt atgtttaatg taaataataa 1080

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<211> 1500
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<213> Artificial

<220>
<223> flanking sequence

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<210> 223
<211> 1323
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<220>
<221> misc_feature
<222> (578)..(578)
<223> n is a, c, g, or t

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<220>
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<400> 224
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ttaaaaaata atccattttt tatcgtcttc catcatttgt ctccagcttt cggagtggaa 240

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<213> Artificial

<220>
<223> flanking sequence

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<212> DNA
<213> Artificial

<220>
<223> flanking sequence

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cgcgagacat atagaacaac acgacgtcgc agtggcatcc ttttgcaagc tataacccac 2940

acgagcctct tcgcacggaa ccttagagca tgcgcaacgg aggatgaccc cgaatcctta 3000

gcgcttaatg gttccattag ttttaatata ataataatat taagcttaat aaacgtaagg 3060

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<211> 1100
<212> DNA
<213> Artificial

<220>
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atgactacaa gtctacaact caactaatca tccacataaa taattagttt tgtcataatt 180

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gacatggaaa aaatcttaga taagaatagt ttgaaattag aaagctcgca attgaggtct 540

accaaaatta gaaattagaa agctcgcaat ccagtcagca tcatcacacc aaaagttagg 600

cccgaatagt ttgaaattag aaagctcgca attgaggtct acaggccaaa ttcgctctta 660

gccgtacaat attactcacc ggtgcgatgc cccccatcgt aggtgaaggt ggaaattaat 720

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<220>
<223> flanking sequence

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acatttttga attgaaaaaa aattggtaat tactctttct ttttctccat attgaccatc 180

atactcattg ctgatccatg tagatttccc ggacatgaag ccatttactc tgaccctact 240

ccacaaatat atttttattt ataaaaaggt ggccattgta tactatgtgt gcgtatacag 300

gaataaaaat gtgtcaatgt atatgtaaac tgattccatc ttatatgtaa tgtgcgtgtg 360

taaatgaaga tactagtatc catgtgtcgc ctacttgatt tgttcaactg taactcataa 420

tatctcaaga ttctttcttt tttttctacg aatatcgcaa tctataatac cattaaatta 480

ttgtaacaaa attggttgac atttataaaa tgaaaaagaa gagaagagca tttaaacacg 540

actgatgaaa gtccaatgta gctagataaa ccacgcgtgg tggtcaatgc gttccattcc 600

aaaaggatcc gagttcgaat ccgcaccaca ccagattttc actgcgcgtg gccatgaagc 660

tttcgcattc tcgctcctga gaatggttct ccatttttt tttccagtgt agctagatac 720

cggtctgaat ctaggtttat aatatgctga caatgtaatg ataattaata catcaaaaca 780

tgtgtttctg aaccaaaata aaaacttttt t 811

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<211> 2900
<212> DNA
<213> Artificial

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<223> flanking sequence

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cctttaaatg agtggaagat gagaaccatg tgatgaaaaa cctgcaaaac aagataaatt 180

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caataaggtt aaatattttt gatcagaaga cttactagac gacttacttg taagtcgccc      420
agaagacttc aatattttta gcgggaaact aaaatatttt tagcgggagt tagaagaccc      480
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gatttattaa caattttaca agagacaagc aaatgtcgag caatcatatt ctataatatc     2280
aacctaaaag agttaaatcc ataaaattag ttggcaacga gcgatagtat gaaagttagg     2340
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tatttaaacc agtcagcatc atcacaccaa aagttaggcc cgaatagttt gaaattagaa 2520

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ataaattttt atatttggta 2900

<210> 231
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<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 231
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atttttgaat tgaaaaaaaa ttggtaatta ctctttcttt ttctccatat tgaccatcat 180

actcattgct gatccatgta gatttcccgg acatgaagcc atttacaatt gaatatatcc 240

tcacatatga aatatatttt ttttttacaa attacaccta ttaaattata cttgatcggt 300

catctgatat atttgaaaga accctatcag ccagctattc ataatttaca taaaagaaaa 360

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aagtaaaatg gagtgtattt taatttttatc ttcagaccaa tgttatcaat gtagcccata 480

tattaatact aaaacaactt ctgcacaaac acacgaatca aagcctcgtg tttcatcgta 540

gctttagcta aaatttccca aaagcaaatt caatagtatt ttactaggtc aaacccacaa 600

gagaaaaaga aagtcaatcc caaggatcaa gaaatgagaa gtgagaggag aatgctttat 660

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acattcatgt ttttactgtt tatacacagt caataattta tacacagctc catgttttaa 840

tatttaccca tctctctttt gtagtctatc gtagactttc acttgtgtcc ccctcatgcg 900

gcaacatcct cagcaacttg atttactata tacaataata caaatcataa gatatttgtt 960

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<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

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ctgaagaaga tgatgtgaat gagatggttg cttggagcaa tgatgaggat gaggaccttg 180

gttggcaaac aaatgaacct ataaggataa ataatgagct caaggtacct atccttcagc 240

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cggtatcata aaataaactc tacctgtgcg ctattgcttg ctaaacctaa aaccattgtg 480

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aaagcatagt cctttggccg aaggttctat cctatggata agtgaaagaa gaacgccatt 600

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acgcaagaaa caagaagaag aaaaacaatg atctcggaac gtctacaagc aatgactcag 960

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<210> 233
<211> 3284
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 233
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ggcctaactt ttggtgtgat gaaagtgtga gaatatttcc atattagaca attctgtaga 180

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<210> 234
<211> 1700
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<213> Artificial

<220>
<223> flanking sequence

<400> 234
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cgacgccgtt tgacctattt tttcttcttc tttgttttct tcctcatttg tattactatt 180

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gaaaccggcg gtaaggatct gagctacaca tgctcaggtt ttttacaacg tgcacaacag 540

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cgttacgtaa gcctacgtag cgtcacgtga gcttagctaa cgctacctag gctcagctga 1620

cgttacgtaa cgctagctag cgtcactcct gcagcaaatt tacacattgc cactaaacgt 1680

ctaaaccctt gtaatttgtt 1700

<210> 235

<211> 1532
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 235
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gcatgtgtag ctcagatcct taccgccggt ttcggttcat tctaatgaat atatcacccg 180

ttactatcgt atttttatga ataatattct ccgttcaatt tactgattgt ctacgtagcg 240

tcacctgacg ttacgtaagg ctacctaggc tcacgtgacg ttacgtaacg ctacgtagcg 300

tcaggtgagg ttagctaacg ctagctagcc tcacctgacg ttaggtaagg ctacgtagcg 360

tcacctgaga ttagctaagc ctacctagac tcacgtgacc ttaggtaacg ctacgtagcg 420

tcaaagcttt acaacgctac acaaaactgc ttgcgtttct tctctcatta cgagaatata 480

ctttttttcaa tattgcttca aaaactttct gtcatgtgaa tttgatttta gatatatgtt 540

taaaaaatac ttcctccccg aaaataagat tttctaaagt gttcgattat taagaattca 600

ttaaatattt ataatttaat ttttctttta ctttattata cattttctaa taacttttta 660

ccaataaaat ttaatcaatt caaatatttt tatttaatgt ttcttaaaag tataaaaaat 720

atcttaatca tatagaaaat atatttttgt ggaacaagta aaaatctaaa acatcttact 780

ttcaggaacg gaggtaatag atagttacat taaaggtctc aacattacta ttttaagtct 840

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ataaaaatat ccaatatatg accttgacca agacaaatgt gctagagtag tattaacggc 960

gtcagttgcc gttaatccca tttaccaaaa tatatatact acaagggaat ctcttctctc 1020

tctctcacca ttcaatcatt tccatctttt tcaaaatctc cttttcccac tctctctctc 1080

tctcgcttga agaacaagat tggtatattt gttacaacgg gctagtttat caatatcttt 1140

cacaaataaa gaagaagaag gtgatatctt tgaggttgat atctcgaaga aaatggggaa 1200

gaaagagcag aaagatcatc attcaacagt agaaagcgac gacaaagtcg aagctgttct 1260

tcatctcctt cgtaaacatt ctcctctcac tcttaaacag gtttgtttac gaattaatta 1320

tctcactgat ctttcattaa tctatcagct ttttttttt gaagtgctgg ttttttatat 1380

tctcgggtta ataaatattg caggagaagt tctgtaacag agcttgtgtc agtagattct 1440

tgagaacaaa aggagacaat gcgaagaaag cagctaaaca gttaaggtca tgcctttcat 1500

ggagatcttc tcttggcatc ggtatactct tc 1532

<210> 236
<211> 1200
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 236
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ctgacgttag gtaaggctac gtagcgtcac ctgagattag ctaagcctac ctagactcac 120

gtgaccttag gtaacgctac gtagcgtcaa agctttacaa cgctacacaa aaatgcttgc 180

gtttcttctc tcattacgag aatatacttt tttcaatatt gcttcaaaaa ctttctgtca 240

tgtgaatttg attttagata tatgtttaaa aaatacttcc tccccgaaaa taagattttc 300

taaagtgttc gattattaag aattcattaa atatttataa tttaattttt cttttacttt 360

attatacatt ttctaataac tttttaccaa taaaatttaa tcaattcaaa tatttttatt 420

taatgtttct taaaagtata aaaaatatct taatcatata gaaaatatat ttttgtggaa 480

caagtaaaaa tctaaaacat cttactttca ggaacggagg taatagatag ttacattaaa 540

ggtctcaaca ttactatttt aagtctattc acttgttttg cttgcttgct aaccaaacaa 600

agtttactag tttaattaat atattaataa aaatatccaa tatatgacct tgaccaagac 660

aaatgtgcta gagtagtatt aacggcgtca gttgccgtta atcccattta ccaaaatata 720

tatactacaa gggaatctct tctctctctc tcaccattca atcatttcca tctttttcaa 780

aatctccttt tcccactctc tctctctctc gcttgaagaa caagattggt atatttgtta 840

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gttgatatct cgaagaaaat ggggaagaaa gagcagaaag atcatcattc aacagtagaa 960

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aaacaggttt gtttacgaat taattatctc actgatcttt cattaatcta tcagcttttt 1080

ttttttgaag tgctggtttt ttatattctc gggttaataa atattgcagg agaagttctg 1140

taacagagct tgtgtcagta gattcttgag aacaaaagga gacaatgcga agaaagcagc 1200

<210> 237
<211> 1600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 237
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actacaaaat ataatagcct caattatctt caaaactaag ttgacattta attatgcttt 120

tgcaagatat ttacttttgt tgttcgatca tatttaatga ttattttggt tttgaaacaa 180

atattaacat tatatatatt gtgtctatat tgaactgttg taaattataa acatcaaaat 240

tttaatgtta tcttaattat aatttctaat actagtatat tcaaaaatca aaataaacat 300

attttataaa atagtgccag tacgtagtat gggagataat actagtggct ttataaaggg 360

aaacattgtc tctaaaatct cagataaaat gttaaaacac acttattcac aattatgaag 420

atttgaaata tctgaaattt caaattgatg cacttggtag aaagcaaagg ttcaacgcta 480

agtctacaag gtgtaataat gaagtgaaaa tgctagttta gattaccctt gatatgtgac 540

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ttaagcacca gaatcaactc gttctccata gagtccagct tttgagatat atgtgaatag 660

ccttgttgca atatacttgt gagtggcagg cgtgatctta ttaacgaaag tccaaattct 720

gaacaaagtt tatatcaagc tacgatggaa atatggaatc cgtatcaaaa tcaactgtac 780

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agaatgaaaa accagcatcc ccaagatgtg gcaccaagtg ctatcgacca caaactacgc 960

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tcagcatcat cacaccaaaa gttaggcccg aatagtttga aattagaaag ctcgcaattg 1080

aggtctacag gccaaattcg ctcttagccg tacaatatta ctcaccggtg cgatgccccc 1140

catcgtaggt gaaggtggaa attaatggcg cgcctgatca ctgattagta actattacgt 1200

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ttgtaatttg tttttgtttt actatgtgtg ttatgtattt gatttgcgat aaatttttat 1440

atttggtact aaatttataa caccttttat gctaacgttt gccaacactt agcaatttgc 1500

aagttgatta attgattcta aattattttt gtcttctaaa tacatatact aatcaactgg 1560

aaatgtaaat atttgctaat atttctacta taggagaatt 1600

<210> 238
<211> 1677
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 238
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cgaatcttgc atggaaaatg ctcttaccat tggtttttaa ttgaaatgtg ctcatatggg 120

ccgtggtttc caaattaaat aaaactacga tgtcatcgag aagtaaaatc aactgtgtcc 180

acattatcag ttttgtgtat acgatgaaat agggtaattc aaaatctagc ttgatatgcc 240

ttttggttca ttttaacctt ctgtaaacat tttttcagat tttgaacaag taaatccaaa 300

aaaaaaaaaa aaaatctcaa ctcaacacta aattatttta atgtataaaa gatgcttaaa 360

acatttggct taaaagaaag aagctaaaaa catagagaac tcttgtaaat tgaagtatga 420

aaatatactg aattgggtat tatatgaatt tttctgattt aggattcaca tgatccaaaa 480

aggaaatcca gaagcactaa tcagacattg gaagtaggat taatcagtga tcagtaacta 540

ttaaattcaa ttaaccgcgg acatctacat ttttgaattg aaaaaaaatt ggtaattact 600

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aatagctcga cattcctttg gtctggtcca gtgatgtcga ctcacaaagc gaagattgca 720

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tgaaagcatc taatgtttat agaaaattac caaaaatacc acatttatga aaaattatca 1140

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ttaacgaaga acaaacgaca tttataatcc taagataatt ttttctaatt caaaaataat 1260

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atatgaaagt tcaaatttga aaaatgataa ttcaaaaaca taaaaaaata tatttttatt 1380

taaataatta tttattatat atatatagac catggagcaa gggggcgtgg gccccggggc 1440

ccaaactttt tttcccatat ataatgtcaa caaggacccg atttttagaa aaaaaaatag 1500

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ttatttgcc caagggccta tagattcatt gggccgaccc tggggttggg gttgtgtgtg 1620

tgtcattcta taattaaccg gcaaaaactc caaaattttt atttaacaaa tgtataa 1677

<210> 239
<211> 5600
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 239
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ttagatgata aattttcatt gtcttgagaa taataaaaaa aaaatctaag gataatatca 120

tagttaaatt tatatgatat ttacttcagt aatattaaaa tattatatac atttttatta 180

tatttggttt agtaatatta aatggattct attttaaatt tcttatgaca atcaaaacca 240

cttagtgtga taatttctga aaaaaattgg caaaaaaatc aaaaatactt atcttattat 300

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ttaataaata attagattaa atttttaata tataattacc catatttaa aacaaatttc 420

attaatttta aaatcatcat tatctaaaaa gattatatat tatgttatcc aaaaatattt 480

tacatcataa tattttaaaa taaatataaa tttatgtata ttgttttatg tatatatgaa 540

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accagcactc taagcaccac 5600

<210> 240
<211> 1321
<212> DNA
<213> Artificial

<220>
<223> flanking sequence

<400> 240
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catcagatac tttcctattt tttggcacct ttcatcggac tactgattta tttcaatgtg 180

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caaaatcctt atttttacag ctttcaaagt ttaacagctg gaaacctgta gaaaatcaga 1020

cacagcctct caagttttct ggacaataaa tactggtaac gtaagaaaac caattaatga 1080

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<210> 241
<211> 100
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 241
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tgacactcaa ctaaaatgtt ggattttccc tttcaggtgt 100

<210> 242
<211> 148
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 242
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aggcctttaa ttaatctaga ggcgcgcc 148

<210> 243
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<212> DNA
<213> Artificial

<220>
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ccgccttcag tttaaactat cagtgttgct ttagtggtca atcaaaagat gaacagtatc 120

ttctcgatcg atgatttctc cgatccc 147

<210> 244
<211> 118
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 244
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aacgacaatc tgatcactga ttagtaacta aggcctttaa ttaatctaga ggcgcgcc 118

<210> 245
<211> 110
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 245
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<210> 246
<211> 95
<212> DNA
<213> Artificial

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<223> amplicon

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tttatattat atacctactt cctctctctc gctct 95

<210> 247
<211> 101
<212> DNA
<213> Artificial

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<400> 247
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acatgaagcc atttgtcaac gtcgttgttt gatttcgtta a 101

<210> 248
<211> 197
<212> DNA
<213> Artificial

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<212> DNA
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<220>

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<210> 250
<211> 137
<212> DNA
<213> Artificial

<220>
<223> amplicon

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tgcttcttca tgcctca 137

<210> 251
<211> 113
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 251
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acatgaagcc atttacaatg agtcaaaaaa aagttgtttt gatgagtcac tgc 113

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<211> 160
<212> DNA
<213> Artificial

<220>
<223> amplicon

<400> 252
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acatgaagcc atttactctg accctactcc acaaatatat ttttatttat aaaaaggtgg 120

ccattgtata ctatgtgtgc gtatacagga ataaaaatgt 160

<210> 253
<211> 169
<212> DNA
<213> Artificial

<220>
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<400> 253
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acaattgaat atatcctcac atatgaaata tatttttttt ttacaaatta cacctattaa 120

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<210> 254
<211> 576
<212> DNA
<213> Artificial

<220>
<223> amplicon

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atatttttt gtacactatt tatcaattta ataaaaaaat ataatataat ttaagatgaa 180

ccaacatatt tctctaaatt ctgaatttta ttttcatggt gacacgtgga tacgaaacaa 240

tgttgtaatg tttatcgatt aatatataat gtatgttata tgtttgttgt actaacaaca 300

agatttcata catttcaaac gaaaaagaga gattgatgtg atcagtatta ttgcttttac 360

aaaactattg aaggttcagc gagttcttag taaagtcgaa tccttgtaat aatctctgtt 420

ccaacgggca aacatgcagc tgttaaacta tcgacgccgt ttgacctatt ttttcttctt 480

ctttgttttc ttcctcattt gtacatgcgt gcaagcctac tgacggttgg atgccttggc 540

acgcacttca acaactctat atccttcctg gtgagg 576

<210> 255
<211> 189
<212> DNA
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<220>
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<400> 255
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gcccgaatag tttgaaatta gaaagctcgc aattgaggtc tacaggccaa attcgctctt 180

agccgtaca 189

<210> 256
<211> 91
<212> DNA
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<220>
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<400> 256
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acacaaaaat gcttgcgttt cttctctcat t 91

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