ILLUMINA, INC.,
Petitioner,

v.

THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK
Patent Owner.

IPR2018-00291, -00318, -00322, -00385, -00797

Patent Owner's Demonstratives

U.S. Patent Nos.
9,718,852 9,719,139 9,708,385 9,725,480 9,868,985

Columbia Exhibit No. 2141
Illumina v. Columbia
IPR2018-00291, 318, 322, 385, 797

TABLE OF CONTENTS

Columbia's Patents

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

Columbia Inventors' Insights

Recognition of Problem & Solution:

Spatial Constraints
(*e.g.*, max. 3.7Å capping group)

Chemical Constraints
(*e.g.*, no ketone, ester, methoxy)

Before 2000

After 2000

Efforts to Develop SBS

Columbia's Invention
(Spatial, Chemical Constraints)

Tsien/Dower Filed

Metzker abstract: 2-nitrobenzyl incorporated

Metzker 1994: 2-nitrobenzyl incorporated

Metzker 1998: 2-nitrobenzyl incorporated

Stemple: reiterating 2-nitrobenzyl incorporated

Dr. Romesberg: "[A] large number of companies and research groups pursued [SBS]"

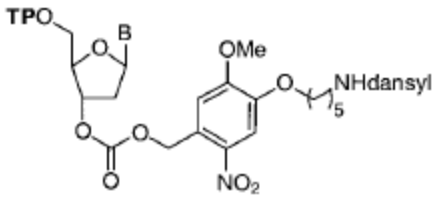
1990 | 1991 | 1992 | 1993 | 1994 | 1995 | 1996 | 1997 | 1998 | 1999 | 2000

Provide no guidance for the capping group
(i) Large groups
(ii) Labels on groups
(iii) Ester; ketone; methoxy

"formidable obstacles"

"major challenge"

Welch testing
(i) Non-ether
(ii) Large group



IPR2018-00291, Sur-Reply, 2-4; PO Response, 7-9

Columbia's Patent Claims Are Narrowly Tailored

'852 patent claim vs. '869 patent claim 16

Narrower terms

New terms

Paper No. _
Filed: October 26, 2018

UNITED STATES PATENT A

BEFORE THE PATENT TRI

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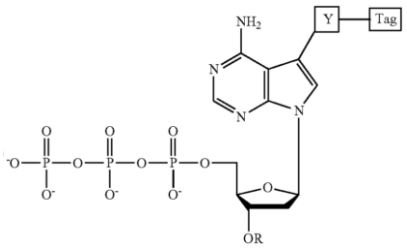
THE TRUSTEES OF CO
IN THE CITY O
Patent

IPR2018-00291 (Pa
IPR2018-00318 (Pa
IPR2018-00322 (Pa
IPR2018-00385 (Pa

PATENT OWNE

[1] An identical Paper is being entered into e

1. An adenine deoxyribonucleotide analogue having the structure:



wherein R (a) represents a small, chemically cleavable, chemical group capping the oxygen at the 3' position of the deoxyribose of the deoxyribonucleotide analogue, (b) does not interfere with recognition of the analogue as a substrate by a DNA polymerase, (c) is stable during a DNA polymerase reaction, and (d) does not contain a ketone group;

wherein OR is not a methoxy group or an ester group;

wherein the covalent bond between the 3'-oxygen and R is stable during a DNA polymerase reaction;

wherein tag represents a detectable fluorescent moiety;

wherein Y represents a chemically cleavable, chemical linker which (a) does not interfere with recognition of the analogue as a substrate by a DNA polymerase and (b) is stable during a DNA polymerase reaction;

and wherein the adenine deoxyribonucleotide analogue:

i) is recognized as a substrate by a DNA polymerase,

ii) is incorporated at the end of a growing strand of DNA during a DNA polymerase reaction,

iii) produces a 3'-OH group on the deoxyribose upon cleavage of R,

iv) no longer includes a tag on the base upon cleavage of Y, and

v) is capable of forming hydrogen bonds with thymine or a thymine nucleotide analogue.

IPR2018-00291, PO Response, 62-66

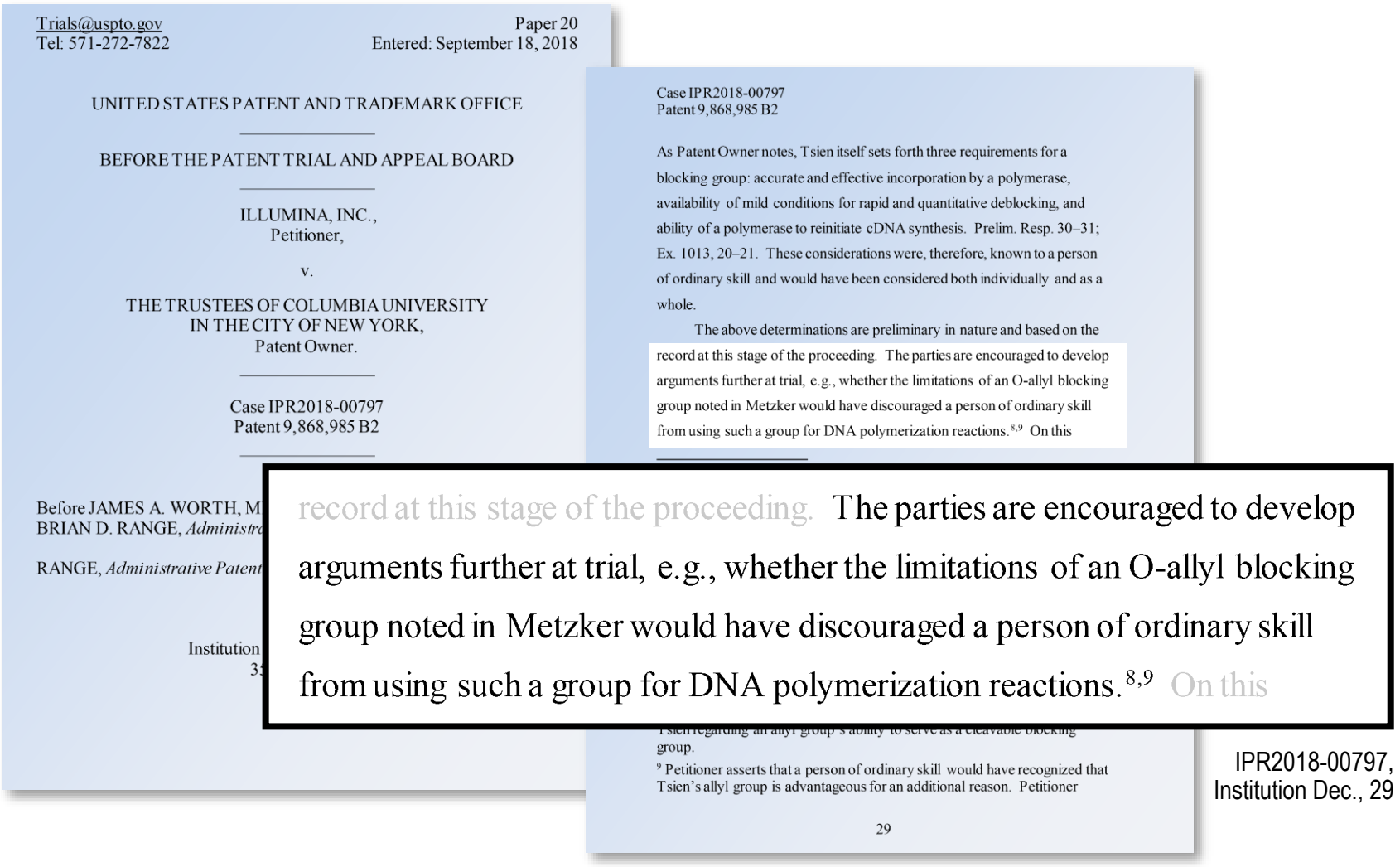
Illumina's Metzker-Based Arguments Fail

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

The Board Requested Additional Briefing Regarding Metzker's Teachings: Metzker Teaches Inefficient 3'-O-Allyl-dATP Incorporation



Trials@uspto.gov
Tel: 571-272-7822

Paper 20
Entered: September 18, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

v.

THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK,
Patent Owner.

Case IPR2018-00797
Patent 9,868,985 B2

Before JAMES A. WORTH, M
BRIAN D. RANGE, *Administra*

RANGE, *Administrative Patent*

Institution
3

Case IPR2018-00797
Patent 9,868,985 B2

As Patent Owner notes, Tsien itself sets forth three requirements for a blocking group: accurate and effective incorporation by a polymerase, availability of mild conditions for rapid and quantitative deblocking, and ability of a polymerase to reinitiate cDNA synthesis. Prelim. Resp. 30–31; Ex. 1013, 20–21. These considerations were, therefore, known to a person of ordinary skill and would have been considered both individually and as a whole.

The above determinations are preliminary in nature and based on the record at this stage of the proceeding. The parties are encouraged to develop arguments further at trial, e.g., whether the limitations of an O-allyl blocking group noted in Metzker would have discouraged a person of ordinary skill from using such a group for DNA polymerization reactions.[8,9] On this

record at this stage of the proceeding. The parties are encouraged to develop arguments further at trial, e.g., whether the limitations of an O-allyl blocking group noted in Metzker would have discouraged a person of ordinary skill from using such a group for DNA polymerization reactions.[8,9] On this

Tsien regarding an allyl group's ability to serve as a cleavable blocking group.

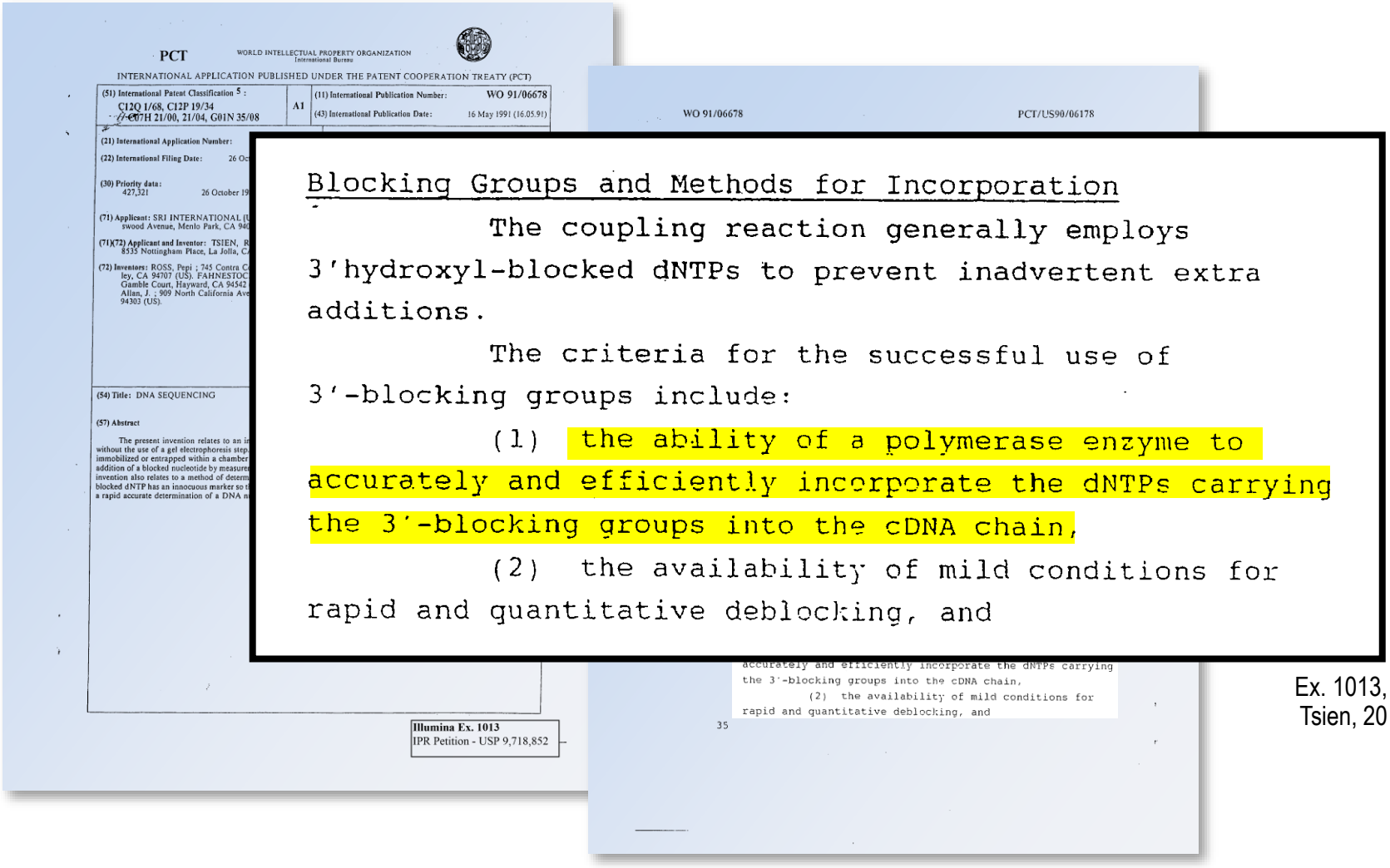
[9] Petitioner asserts that a person of ordinary skill would have recognized that Tsien's allyl group is advantageous for an additional reason. Petitioner

29

IPR2018-00797, Institution Dec., 29

IPR2018-00291, PO Response, 2, 13

No Dispute that SBS Requires Efficient Incorporation



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5 : C12Q 1/68, C12P 19/34, C07H 21/00, 21/04, G01N 35/08 — A1

(11) International Publication Number: WO 91/06678

(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number:

(22) International Filing Date: 26 Oc

(30) Priority data: 427,321 26 October 19

(71) Applicant: SRI INTERNATIONAL [U... Swood Avenue, Menlo Park, CA 940...

(71)(72) Applicant and Inventor: TSIEN, R... 8535 Nottingham Place, La Jolla, C...

(72) Inventors: ROSS, Pepi ; 745 Contra C... ley, CA 94707 (US). FAHNESTOC... Gamble Court, Hayward, CA 94542 ... Allen, J. ; 909 North California Ave... 94303 (US).

(54) Title: DNA SEQUENCING

(57) Abstract

The present invention relates to an i... without the use of a gel electrophoresis step... immobilized or entrapped within a chamber... addition of a blocked nucleotide by measure... invention also relates to a method of determ... blocked dNTP has an innocuous marker so t... a rapid accurate determination of a DNA n...

Illumina Ex. 1013
IPR Petition - USP 9,718,852

WO 91/06678 PCT/US90/06178

Blocking Groups and Methods for Incorporation

The coupling reaction generally employs 3'hydroxyl-blocked dNTPs to prevent inadvertent extra additions.

The criteria for the successful use of 3'-blocking groups include:

(1) the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs carrying the 3'-blocking groups into the cDNA chain,

(2) the availability of mild conditions for rapid and quantitative deblocking, and

accurately and efficiently incorporate the dNTPs carrying the 3'-blocking groups into the cDNA chain,

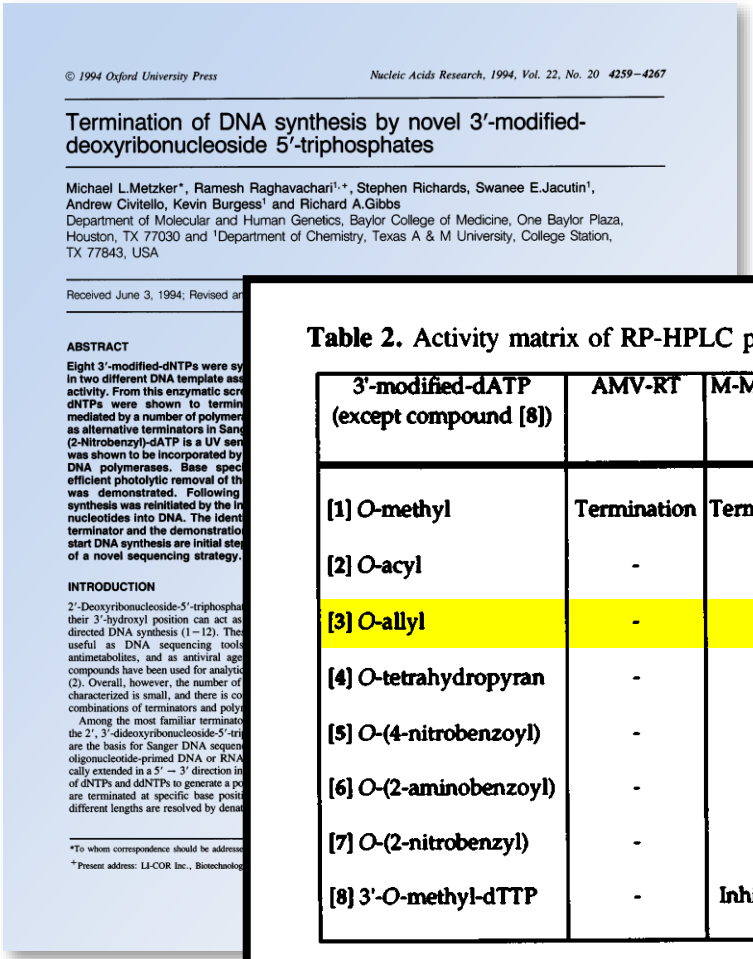
(2) the availability of mild conditions for rapid and quantitative deblocking, and

35

Ex. 1013, Tsien, 20

IPR2018-00291, PO Response, 5-7

Metzker 1994 Taught Termination*



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Nucleic Acids Research, 1994, Vol. 22, No. 20 4259–4267

Termination of DNA synthesis by novel 3′-modified-deoxyribonucleoside 5′-triphosphates

Michael L.Metzker*, Ramesh Raghavachari[1,+], Stephen Richards, Swanee E.Jacutin[1], Andrew Civitello, Kevin Burgess[1] and Richard A.Gibbs

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 and [1]Department of Chemistry, Texas A & M University, College Station, TX 77843, USA

Received June 3, 1994; Revised ar

ABSTRACT

Eight 3′-modified-dNTPs were sy in two different DNA template ass activity. From this enzymatic scr dNTPs were shown to termin mediated by a number of polymer as alternative terminators in San (2-Nitrobenzyl)-dATP is a UV sen was shown to be incorporated by DNA polymerases. Base spec efficient photolytic removal of th was demonstrated. Following synthesis was reinitiated by the in nucleotides into DNA. The ident terminator and the demonstratio start DNA synthesis are initial ste of a novel sequencing strategy.

INTRODUCTION

2′-Deoxyribonucleoside-5′-triphospha their 3′-hydroxyl position can act as directed DNA synthesis (1–12). The useful as DNA sequencing tools antimetabolites, and as antiviral age compounds have been used for analytic (2). Overall, however, the number of characterized is small, and there is co combinations of terminators and poly

Among the most familiar terminato the 2′, 3′-dideoxyribonucleoside-5′-tri are the basis for Sanger DNA sequen oligonucleotide-primed DNA or RNA cally extended in a 5′ → 3′ direction in of dNTPs and ddNTPs to generate a po are terminated at specific base posit different lengths are resolved by denat

*To whom correspondence should be addresse

+Present address: LI-COR Inc., Biotechnolog

Table 2. Activity matrix of RP-HPLC purified 3′-protecting dNTPs challenged against commercially available polymerases

3′-modified-dATP (except compound [8])	AMV-RT	M-MuLV-RT	Klenow fragment	Sequenase®	*Bst* DNA polymerase	AmpliTaq® DNA polymerase	Vent$_R$(exo$^-$)® DNA polymerase	*rTth* DNA polymerase
[1] *O*-methyl	Termination	Termination*	-	-	-	-	Inhibition	Inhibition*
[2] *O*-acyl	-	-	-	-	-	-	Inhibition	-
[3] *O*-allyl	-	-	-	-	-	-	Termination*	-
[4] *O*-tetrahydropyran	-	-	-	-	-	-	-	-
[5] *O*-(4-nitrobenzoyl)	-	-	-	-	-	-	-	-
[6] *O*-(2-aminobenzoyl)	-	-	-	-	-	-	-	-
[7] *O*-(2-nitrobenzyl)	-	-	-	Inhibition	Termination	Termination*	Termination*	-
[8] 3′-*O*-methyl-dTTP	-	Inhibition	-	Inhibition	Termination	Termination	Termination	Termination

All compounds were assayed at a final concentration of 250 μM according to the conditions specified in Table 1. '–' means no activity was detected, 'Termination' means that the termination bands mimic ddNTP termination bands, and 'Inhibition' means the rate of DNA synthesis is reduced in a nonspecific manner. '*' means the activity was incomplete at a final concentration of 250 μM .

Ex. 1016, Metzker (1994), 4263

Termination* Results From Inefficient Incorporation

these nucleotides (10). We note that 3′-*O*-allyl-dATP is not efficiently incorporated by Vent(exo⁻) polymerase (46). Ju and colleagues have reported, however, that

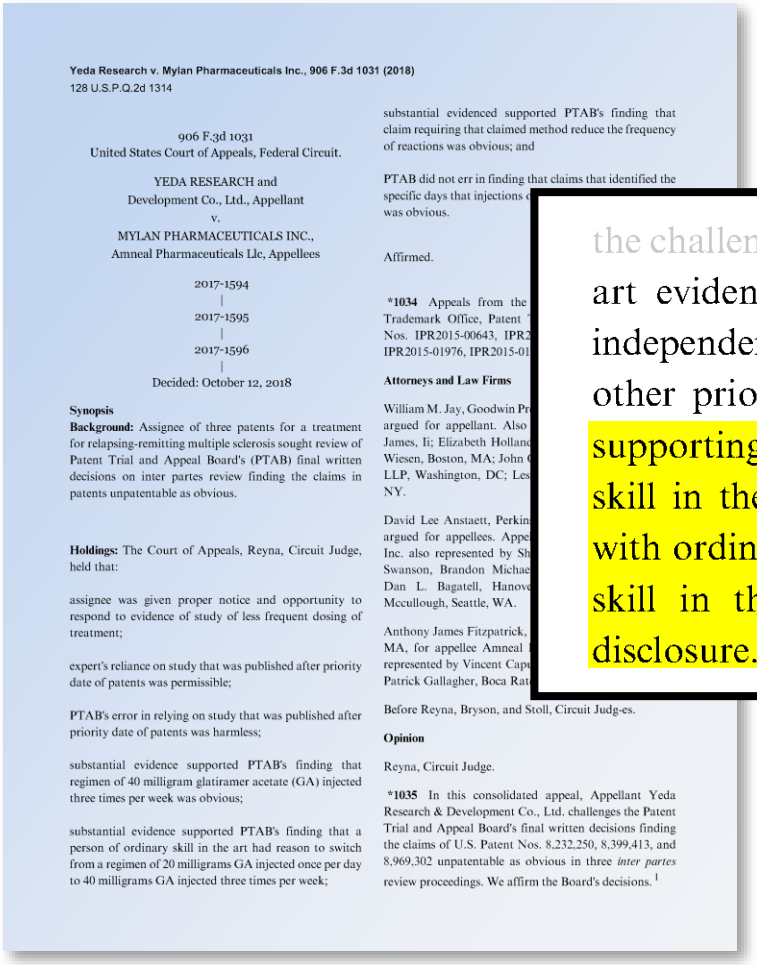
DNA polymerases. This is unlike the situation for 3′-modified nucleotides, which typically act as poor substrates for DNA polymerases. For example, screening 3′-*O*-allyl-dATP with eight different DNA polymerases revealed limited activity at high micromolar concentrations with only Vent(exo−) DNA polymerase (8). The

Ex. 2025, Metzker (2011), 10

Ex. 1017, Metzker (2007), 6348

IPR2018-00291, PO Response, 22

Columbia's Use of Metzker's Later Papers Is Proper



Yeda Research v. Mylan Pharmaceuticals Inc., 906 F.3d 1031 (2018)
128 U.S.P.Q.2d 1314

906 F.3d 1031
United States Court of Appeals, Federal Circuit.

YEDA RESEARCH and
Development Co., Ltd., Appellant
v.
MYLAN PHARMACEUTICALS INC.,
Amneal Pharmaceuticals Llc, Appellees

2017-1594
|
2017-1595
|
2017-1596
|
Decided: October 12, 2018

Synopsis
Background: Assignee of three patents for a treatment for relapsing-remitting multiple sclerosis sought review of Patent Trial and Appeal Board's (PTAB) final written decisions on inter partes review finding the claims in patents unpatentable as obvious.

Holdings: The Court of Appeals, Reyna, Circuit Judge, held that:

assignee was given proper notice and opportunity to respond to evidence of study of less frequent dosing of treatment;

expert's reliance on study that was published after priority date of patents was permissible;

PTAB's error in relying on study that was published after priority date of patents was harmless;

substantial evidence supported PTAB's finding that regimen of 40 milligram glatiramer acetate (GA) injected three times per week was obvious;

substantial evidence supported PTAB's finding that a person of ordinary skill in the art had reason to switch from a regimen of 20 milligrams GA injected once per day to 40 milligrams GA injected three times per week;

substantial evidenced supported PTAB's finding that claim requiring that claimed method reduce the frequency of reactions was obvious; and

PTAB did not err in finding that claims that identified the specific days that injections c
was obvious.

Affirmed.

***1034** Appeals from the
Trademark Office, Patent
Nos. IPR2015-00643, IPR2
IPR2015-01976, IPR2015-01

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NY.

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Dan L. Bagatell, Hanov
Mccullough, Seattle, WA.

Anthony James Fitzpatrick,
MA, for appellee Amneal
represented by Vincent Capu
Patrick Gallagher, Boca Rat

Before Reyna, Bryson, and Stoll, Circuit Judg-es.

Opinion

Reyna, Circuit Judge.

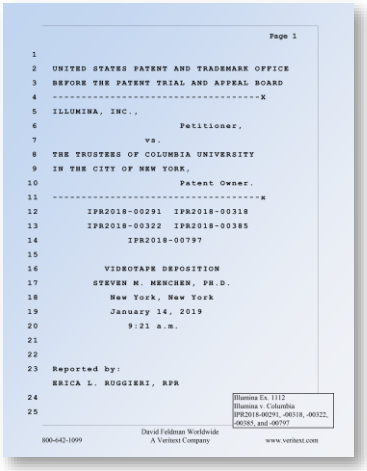
***1035** In this consolidated appeal, Appellant Yeda Research & Development Co., Ltd. challenges the Patent Trial and Appeal Board's final written decisions finding the claims of U.S. Patent Nos. 8,232,250, 8,399,413, and 8,969,302 unpatentable as obvious in three *inter partes* review proceedings. We affirm the Board's decisions. [1]

the challenge"). The Board has recognized that non-prior art evidence of what was known "cannot be applied, independently, as teachings separately combinable" with other prior art, but "can be relied on for their proper supporting roles, e.g., indicating the level of ordinary skill in the art, what certain terms would mean to one with ordinary skill in the art, and how one with ordinary skill in the art would have under-stood a prior art disclosure." *Dominion Dealer Sols., LLC v. AutoAlert,*

Yeda Res. v. Mylan Pharm.,
906 F.3d 1031, 1041 (Fed. Cir. 2018)
(internal citations omitted)

IPR2018-00291, PO Response, 22

Illumina's Contention That SBS Requirements Changed Over Time Is Belied by the Evidence



Page 1

UNITED STATES PATENT AND TRADEMARK OFFICE
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Patent Owner.

IPR2018-00291 IPR2018-00318
IPR2018-00322 IPR2018-00385
IPR2018-00797

VIDEOTAPE DEPOSITION
STEVEN M. MENCHEN, PH.D.
New York, New York
January 14, 2019
9:21 a.m.

Reported by:
ERICA L. RUGGIERI, RPR

Illumina Ex. 1112
Illumina v. Columbia
IPR2018-00291, -00318, -00322, -00385, and -00797

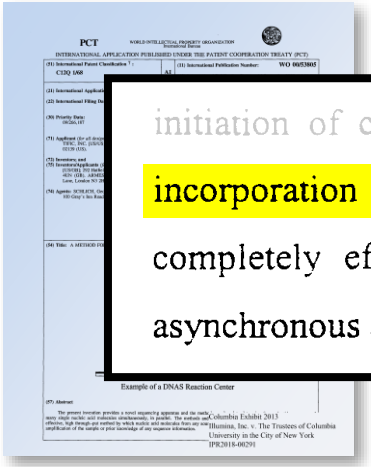
800-642-1099 David Feldman Worldwide A Veritext Company www.veritext.com

Stephen Menchen, Ph.D.
Deposition

Q. You are aware that standards for what's efficient incorporation of DNA analogs changes over time as technology improves?

A. Yeah, I -- I understand what you are asking. I can't think of a specific example of that happening.

Ex. 1113, Menchen Dep., 369:21-370:6



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)
(51) International Patent Classification: C12Q 1/68 A1
(11) International Publication Number: WO 00/53805

Example of a DNAS Reaction Center

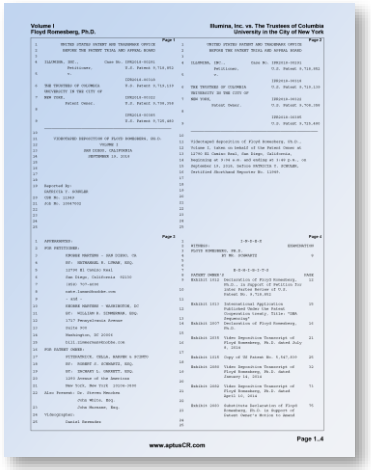
Columbia Exhibit 2013
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291

initiation of chain growth. Moreover, this technique is plagued by any inefficiencies of incorporation and deprotection. Because incorporation and 3'-OH regeneration are not completely efficient, a pool of initially identical extending strands can rapidly become asynchronous and sequences cannot be resolved beyond a few limited initial additions.

Ex. 2013, Stemple (2000), 2-3

IPR2018-00291, Sur-Reply, 9

Metzker Did Not Teach That the 3'-O-Allyl-dATP Had Useful Properties as an Alternative Terminator



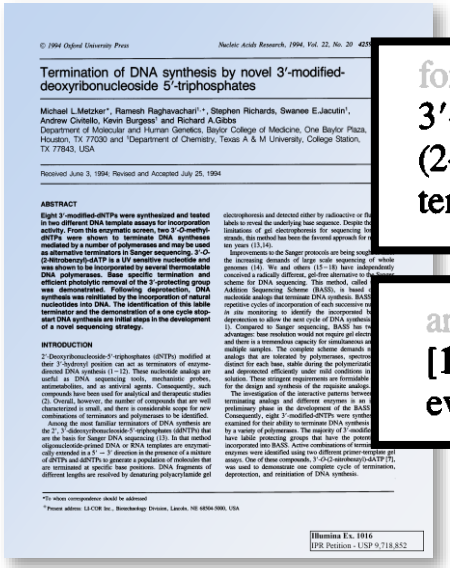
Floyd Romesberg, Ph.D.
Deposition

Q. Metzker does not say that the 3'-O-allyl dNTP was shown to terminate DNA synthesis and may be used as an alternative terminator in Sanger sequencing, right?

A. He does not say that.

Ex. 2126, Romesberg Dep., 106:10-107:2

Metzker Abandoned the 3'-O-Allyl-dATP



Termination of DNA synthesis by novel 3′-modified-deoxyribonucleoside 5′-triphosphates

Michael L.Metzker*, Ramesh Raghavachari[+], Stephen Richards, Swanee E.Jacutin[+], Andrew Civitello, Kevin Burgess[+] and Richard A.Gibbs

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 and [+]Department of Chemistry, Texas A & M University, College Station, TX 77843, USA

Received June 3, 1994; Revised and Accepted July 25, 1994

ABSTRACT

Eight 3′-modified-dNTPs were synthesized and tested in two different DNA template assays for incorporation activity. From this enzymatic screen, two 3′-*O*-methyl-dNTPs were shown to terminate DNA syntheses mediated by a number of polymerases and may be used as alternative terminators in Sanger sequencing. 3′-*O*-(2-Nitrobenzyl)-dATP is a UV sensitive nucleotide and was shown to be incorporated by several thermostable DNA polymerases. Base specific termination and efficient photolytic removal of the 3′-protecting group was demonstrated. Following deprotection, DNA synthesis was reinitiated by the incorporation of natural nucleotides into DNA. The identification of this labile terminator and the demonstration of a one cycle stop-start DNA synthesis are initial steps in the development of a novel sequencing strategy.

INTRODUCTION

2′-Deoxyribonucleoside-5′-triphosphates (dNTPs) modified at their 3′-hydroxyl position can act as terminators of enzyme-directed DNA synthesis (1–12). These nucleotide analogs are useful as DNA sequencing tools, mechanistic probes, antimetabolites, and as antiviral agents. Consequently, such compounds have been used for analytical and therapeutic studies (2). Overall, however, the number of compounds that are well characterized is small, and there is considerable scope for new combinations of terminators and polymerases to be identified.

Among the most familiar terminators of DNA synthesis are the 2′, 3′-dideoxyribonucleoside-5′-triphosphates (ddNTPs) that are the basis for Sanger DNA sequencing (13). In that method oligonucleotide-primed DNA or RNA templates are enzymatically extended in a 5′ → 3′ direction in the presence of a mixture of dNTPs and ddNTPs to generate a population of molecules that are terminated at specific base positions. DNA fragments of different lengths are resolved by denaturing polyacrylamide gel electrophoresis and detected either by radioactive or fluorescent labels to reveal the underlying base sequence. Despite the limitations of gel electrophoresis for sequencing long strands, this method has been the favored approach for ten years (13,14).

Improvements to the Sanger protocols are being sought to meet the increasing demands of large scale sequencing of whole genomes (14). We and others (15–18) have independently conceived a radically different, gel-free alternative to the Sanger scheme for DNA sequencing. This method, called Base Addition Sequencing Scheme (BASS), is based on a single nucleotide analog that terminates DNA synthesis. BASS uses repetitive cycles of incorporation of each successive nucleotide in situ monitoring to identify the incorporated base, and deprotection to allow the next cycle of DNA synthesis (Figure 1). Compared to Sanger sequencing, BASS has two major advantages: base resolution would not require gel electrophoresis and there is a tremendous capacity for simultaneous analysis of multiple samples. The complete scheme demands the use of analogs that are tolerated by polymerases, spectroscopically distinct for each base, stable during the polymerization cycle, and deprotected efficiently under mild conditions in aqueous solution. These stringent requirements are formidable challenges for the design and synthesis of the required nucleotide analogs.

The investigation of the interactive patterns between DNA terminating analogs and different enzymes is an important preliminary phase in the development of the BASS strategy. Consequently, eight 3′-modified-dNTPs were synthesized and examined for their ability to terminate DNA synthesis mediated by a variety of polymerases. The majority of 3′-modified-dNTPs have labile protecting groups that have the potential to be incorporated into BASS. Active combinations of terminator and polymerase enzymes were identified using two different primer-template gel assays. One of these compounds, 3′-*O*-(2-nitrobenzyl)-dATP (7), was used to demonstrate one complete cycle of termination, deprotection, and reinitiation of DNA synthesis.

*To whom correspondence should be addressed

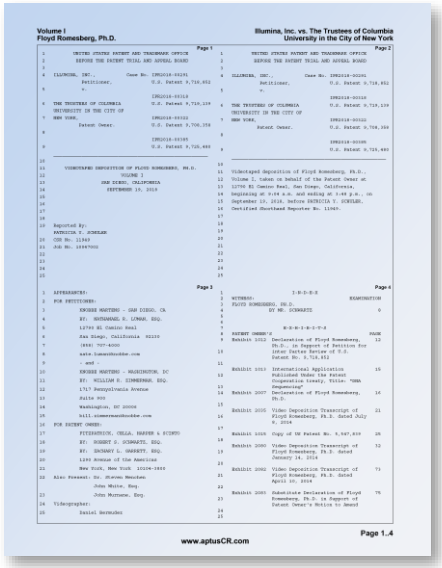
[+]Present address: LI-COR Inc., Biotechnology Division, Lincoln, NE 68504-3000, USA

Illumina Ex. 1016
IPR Petition - USP 9,718,852

for incorporation by a variety of polymerases. **Three compounds, 3′-*O*-methyl-dATP [1], 3′-*O*-methyl-dTTP [8], and 3′-*O*-(2-nitrobenzyl)-dATP [7], proved to be interesting DNA synthesis terminators.** In Sanger sequencing, the 3′-*O*-methyl analogs

an alteration in enzymatic fidelity is discussed below. **Compounds [1], [8], and [7] showed specific termination and were further evaluated with respect to their concentration dependent effects.**

Ex. 1016, Metzker (1994), 4263, 4265



Volume I
Floyd Romesberg, Ph.D.

Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC., Petitioner, v. THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK, Patent Owner.

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.
VOLUME I
SAN DIEGO, CALIFORNIA
SEPTEMBER 19, 2018

Reported By:
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John Mortensen, Esq.

Videographer:
Daniel Hernandez

Videotaped deposition of Floyd Romesberg, Ph.D., Volume I, taken on behalf of the Patent Owner at 12790 El Camino Real, San Diego, California, beginning at 9:04 a.m. and ending at 5:48 p.m. on September 19, 2018, before PATRICIA F. SCHELER, Certified Shorthand Reporter No. 11569.

EXHIBITS

PATENT OWNER'S — PAGE
Exhibit 1012 Declaration of Floyd Romesberg, Ph.D., in Support of Petition for Inter Partes Review of U.S. Patent No. 9,718,852 — 12
Exhibit 1013 International Application Published Under the Patent Cooperation Treaty, Title: "DNA Sequencing" — 15
Exhibit 2007 Declaration of Floyd Romesberg, Ph.D. — 16
Exhibit 2035 Video Deposition Transcript of Floyd Romesberg, Ph.D. Dated July 9, 2019 — 21
Exhibit 2036 Copy of US Patent No. 9,567,819 — 26
Exhibit 2060 Video Deposition Transcript of Floyd Romesberg, Ph.D. Dated January 14, 2019 — 32
Exhibit 2062 Video Deposition Transcript of Floyd Romesberg, Ph.D. Dated April 10, 2019 — 73
Exhibit 2065 Substitute Declaration of Floyd Romesberg, Ph.D. in Support of Patent Owner's Motion to Amend — 76

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Page 1..4

Floyd Romesberg, Ph.D.
Deposition

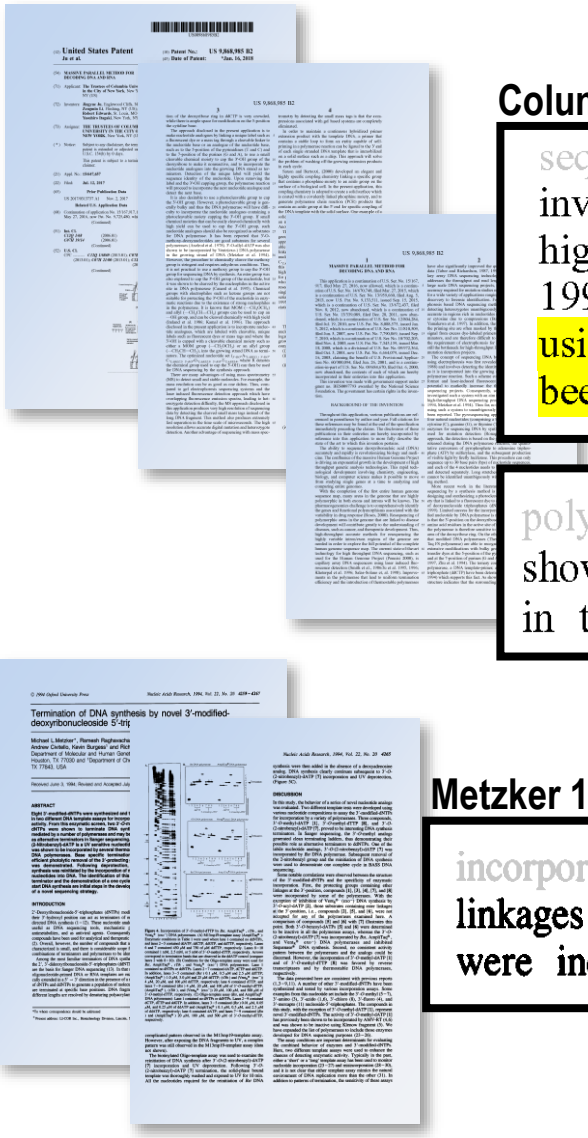
Q. But he didn't move [3'-O-allyl-dATP] forward?

A. He did not move [3'-O-allyl-dATP] forward.

Ex. 2126, Romesberg Dep.,132:1-2

IPR2018-00291, PO Response, 23

Columbia's Patent "Admission" Says Nothing More Than Metzker Itself Says about the 3'-O-Allyl-dATP



Columbia Patent

sequencing projects. Consequently, several groups have investigated such a system with an aim to construct an ultra high-throughput DNA sequencing procedure (Cheeseman 1994, Metzker et al. 1994). Thus far, no complete success of using such a system to unambiguously sequence DNA has been reported. The pyrosequencing approach that employs

polymerases (Axelrod et al. 1978). 3'-O-allyl-dATP was also shown to be incorporated by Ventr(exo-) DNA polymerase in the growing strand of DNA (Metzker et al. 1994).

Ex. 1075, '985 Patent, 2:27-32, 3:28-30*

Metzker 1994

incorporation. **First, the protecting groups containing ether linkages at the 3′-position, compounds [1], [3], [4], [7], and [8] were incorporated by some of the polymerases.** With the

Ex. 1016, Metzker (1994), at 4265

* References incorporated in their entirety, Ex. 1075, 1:35-38

Illumina's Solexa Patent Application Credits Columbia With Discovering -CH_2-CH=CH_2 Capping Group

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WO 2004/018497 A2

(54) Title: MODIFIED NUCLEOTIDES

(57) Abstract: The invention provides modified nucleotide or nucleoside mo... ribose or deoxyribose sugar moiety having a removable 3'-OH blocking group... atom has attached a group of the structure -O-Z wherein Z is any of -C(R")2-O-... and -C(R")2-F, wherein each R" is or is part of a removable protecting group; ... substituted alkyl, arylalkyl, alkenyl, alkynyl, aryl, heteroaryl, heterocyclic, a... group, or a detectable label attached through a linking group, or (R")2 represe... each R" may be the same or different and is selected from the group comprisi... wherein said molecule may be reacted to yield an intermediate in which each ... F is exchanged for OH, SH or NH2, preferably OH, which intermediate disso... with a free 3'OH; with the proviso that where Z is -C(R")2-S-R", both R" grou...

Illumina Ex. 11...
IPR2018-0029...
and -00797

blocking group will therefore exhibit long term stability, be ef... enzyme, cause to... incorporation ar... mild conditions ... polynucleotide s... conditions. Thes... obstacles to the... modified nucleot...

Reversible ... been described p... the above criter... compatible, chemistry.

Metzker *et al.*, (*Nucleic Acids Research*, 22(20): 4259-4267, 1994) discloses the synthesis and use of eight 3'-modified 2-deoxyribonucleoside 5'-triphosphates (3'-modified dNTPs) and testing in two DNA template assays for incorporation activity. The 3'-modified dNTPs included 3'allyl deoxyriboadenosine 5'-triphosphate (3'-allyl dATP). However, the 3'allyl blocked compound was not used to demonstrate a complete cycle of termination, deprotection and reinitiation of DNA synthesis: the only test results presented were those which showed the ability of this compound to terminate DNA synthesis in a single termination assay, out of eight such assays conducted, each conducted with a different DNA polymerase.

WO02/29003 (The Trustees of Columbia University in the City of New York) describes a sequencing method which may include the use of an allyl protecting group to cap the 3'-OH group on a growing strand of DNA in a polymerase reaction. The allyl group is introduced according to the procedure of Metzker (*infra*) and is said

WO02/29003 (The Trustees of Columbia University in the City of New York) describes a sequencing method which may include the use of an allyl protecting group to cap the 3'-OH group on a growing strand of DNA in a polymerase reaction. The allyl group is introduced according to the procedure of Metzker (*infra*) and is said

Ex. 1125, 3

Dr. Romesberg in 2005 Credited Columbia With Pioneering the Allyl-Capping Group

Principal Investigator/Program Director (Last, First, Middle): **Romesberg, Floyd E.**

A. Specific Aims

This R21 submission is in response to PA-03-058, 'Exploratory/Developmental Bioengineering Research Grants.' We propose the directed evolution of new DNA polymerases that will facilitate several emerging sequencing technologies. After successful completion of the proposed research we will seek additional funding to develop the most promising applications.

There is great incentive for developing low cost technologies for sequencing genomes. During the past two decades, along with computational advances, the parallelization, automation, and refinement of the Sanger method has reduced sequencing costs from tens of dollars per sequenced base to hundredths of a cent per sequenced base. However, the cost of sequencing a mammalian-sized genome is still tens of millions of dollars, and it is unlikely that further advances in instrumentation alone will achieve the 10,000-fold reduction in cost set as a goal by the NIH.

A variety of new sequencing methods currently in development have the potential to make genome sequencing commonplace. Most of these new methods rely on polymerase mediated DNA synthesis with modified deoxynucleotide triphosphate (dNTP) substrates. As a result, these DNA sequencing methods are all limited by the ability of the available polymerases to recognize modified dNTPs. Despite obvious limitations with the polymerases, and in contrast to the great effort devoted towards improvements in automation and new technique development, little effort has been directed toward using the modern methods of directed evolution to evolve enzymes that are better suited to sequencing.

We have developed an activity-based selection system to evolve polymerases with novel activities (Figure 1). As described in detail below, the selection system involves the co-display on phage of DNA polymerase libraries and an oligonucleotide substrate that primes synthesis with an attached template. If a displayed polymerase mutant recognizes the attached substrate and the provided dNTPs, then it will extend the primer, and only then covalently attach a biotin-dUTP. Biotinylated phage particles may be selectively recovered using a streptavidin solid support. We have successfully used the system to evolve several polymerases that catalyze unnatural reactions with efficiencies that rival those of natural polymerases with their native

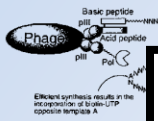


Figure 1. Phage particle with attac
substrate. X represents a natura
For clarity, only two pIII proteins ar
are designed so that biotin incorpo
on DNA synthesis with natural or m

substrates [1,2]. We are now inte
system to evolve polymerases th
improve different sequencing m
proposal focuses on evolving the
Thermus aquaticus polymerase I
fragment of *E. coli* DNA Polymer
as a backup in the event that u
arise with Sf. The specific aims of this proposal are:

Specific Aim 1, Year 1: polymerases that efficie
DNA using Cy5-labeled d
novel synthes
methodologies.

Specific Aim 2, Years polymerases that efficien
reversibly fluorophore tagg
3′-*O*-allyl protecting gro
continue synthesis after
allyl group removal.

Specific Aim 3, Year 2: Collaborate to evaluate evolved polymerases in actual sequencing applications.

The overall goal of the proposed research is the directed evolution of DNA polymerases to enable revolutionary sequencing methodologies. To facilitate the last specific aim, we have established collaborations with Prof. Steve Quake (Stanford), Prof. Jingyue Ju (Columbia), and Dr. Phillip Ordoukhanian, director of the Scripps sequencing facility.

B. Background and Significance

Currently, Sanger dideoxy sequencing and capillary electrophoresis is the most well-established and popular technique employed for DNA sequencing.

PHS 398/2590 (Rev. 09/04) Page 22 Continuation Format Page

Page 27 of 50

Principal Investigator/Program Director (Last, First, Middle): **Romesberg, Floyd E.**

Columbia has reported a set of four dyes, one linked to each dNTP by a photolabile group that were

Thermo Sequenase. Additionally, the approach was

concentrations and extended incubation periods to drive extension of the 'deprotected' primer termini.

The Ju group has pioneered the development of novel dNTPs that not only bear photo-detachable nucleobase-linked fluorophores (see above), but that also bear 3′-OH protecting groups. First proposed by Metzker in 1994 [10], reversible modification of the 3′-OH allows for strict control over the synthesis beyond the modified nucleotide since the required nucleophile is not available until deprotection. When optimized, 3′ modification should allow all four labeled dNTPs to be present in a given reaction, as opposed to flushing the system with solutions containing only one labeled dNTP at a time. In addition, these protecting groups would prevent multiple incorporations of the same dNTP (modified or unmodified) at homopolymeric runs. Specifically, the Ju group has developed 3′-*O*-allyl protecting groups that may be catalytically removed with palladium [11]. However, like all 3′-*O*-modified dNTPs examined, as well as the nucleobase-modified dNTPs discussed above, these 3′-protected dNTPs are very inefficient substrates

Function.

Many 'Type I' (or 'A family') polymerases have played an important role in sequencing efforts, including both Taq (from which Sf is derived) and Kf [12]. Careful consideration of the structure and substrate repertoire of these polymerases is expected to facilitate their directed evolution.

(B.3.1) *Type I DNA Polymerases Structure and Mechanism.* In addition to being the polymerases of choice for sequencing, Type I DNA polymerases are an attractive starting point for directed evolution experiments due to their small size, thorough characterization [13], and structural and functional homology [14,15].

Crystal structure data for each of these type I polymerases shows a structure resembling a right-hand with 'finger,' 'palm,' and 'thumb' subdomains forming a deep substrate binding cleft (Figure 2). The duplex DNA approaches the polymerase domain from the 3′-5′ exonuclease side of the cleft and binds with the primer terminus in the polymerase active site, packed against the N-

PHS 398/2590 (Rev. 09/04) Page 24 Continuation Format Page

Page 29 of 50

The Ju group has pioneered the development of novel dNTPs that not only bear photo-detachable nucleobase-linked fluorophores (see above), but that also bear 3′-OH protecting groups. First proposed

Specifically, the Ju group has developed 3′-*O*-allyl protecting groups that may be catalytically removed with palladium [11]. However, like all 3′-*O*-

Ex. 2118, Romesberg NIH Grant, 29

IPR2018-00291, PO Response, 28-29

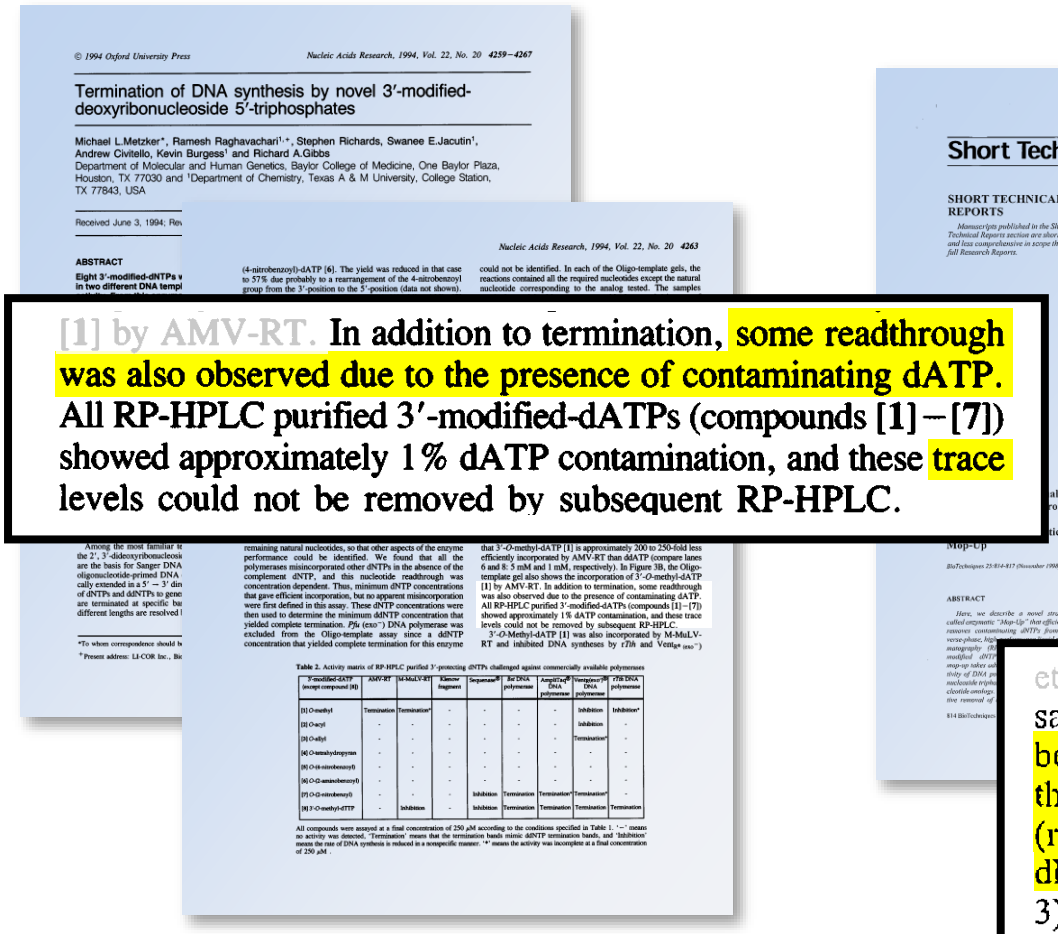
Illumina's Belated Contamination, High Concentration, and Vent Polymerase Variant Theories Fail

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

Contamination Did Not Cause Metzker's Termination*: Metzker's Assays Differentiate Incorporation and Contamination



© 1994 Oxford University Press — Nucleic Acids Research, 1994, Vol. 22, No. 20 4259–4267

Termination of DNA synthesis by novel 3′-modified-deoxyribonucleoside 5′-triphosphates

Michael L.Metzker*, Ramesh Raghavachari[1,+], Stephen Richards, Swanee E.Jacutin[1], Andrew Civitello, Kevin Burgess[1] and Richard A.Gibbs

Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX 77030 and [1]Department of Chemistry, Texas A & M University, College Station, TX 77843, USA

Received June 3, 1994; Rev

ABSTRACT

Eight 3′-modified-dNTPs v
in two different DNA templ

Among the most familiar t
the 2′, 3′-dideoxyribonucleos
are the basis for Sanger DNA
oligonucleotide-primed DNA
cally extended in a 5′ → 3′ din
of dNTPs and ddNTPs to gene
are terminated at specific ba
different lengths are resolved

*To whom correspondence should b
+Present address: LI-COR Inc., Bi

Nucleic Acids Research, 1994, Vol. 22, No. 20 4263

(4-nitrobenzoyl)-dATP [6]. The yield was reduced in that case to 57% due probably to a rearrangement of the 4-nitrobenzoyl group from the 3′-position to the 5′-position (data not shown).

could not be identified. In each of the Oligo-template gels, the reactions contained all the required nucleotides except the natural nucleotide corresponding to the analog tested. The samples

remaining natural nucleotides, so that other aspects of the enzyme performance could be identified. We found that all the polymerases misincorporated other dNTPs in the absence of the complement dNTP, and this nucleotide readthrough was concentration dependent. Thus, minimum dNTP concentrations that gave efficient incorporation, but no apparent misincorporation were first defined in this assay. These dNTP concentrations were then used to determine the minimum dNTP concentration that yielded complete termination. *Pfu* (exo⁻) DNA polymerase was excluded from the Oligo-template assay since a ddNTP concentration that yielded complete termination for this enzyme

that 3′-O-methyl-dATP [1] is approximately 200 to 250-fold less efficiently incorporated by AMV-RT than dATP (compare lanes 6 and 8: 5 mM and 1 mM, respectively). In Figure 3B, the Oligo-template gel also shows the incorporation of 3′-O-methyl-dATP [1] by AMV-RT. In addition to termination, some readthrough was also observed due to the presence of contaminating dATP. All RP-HPLC purified 3′-modified-dATPs (compounds [1]–[7]) showed approximately 1% dATP contamination, and these trace levels could not be removed by subsequent RP-HPLC.

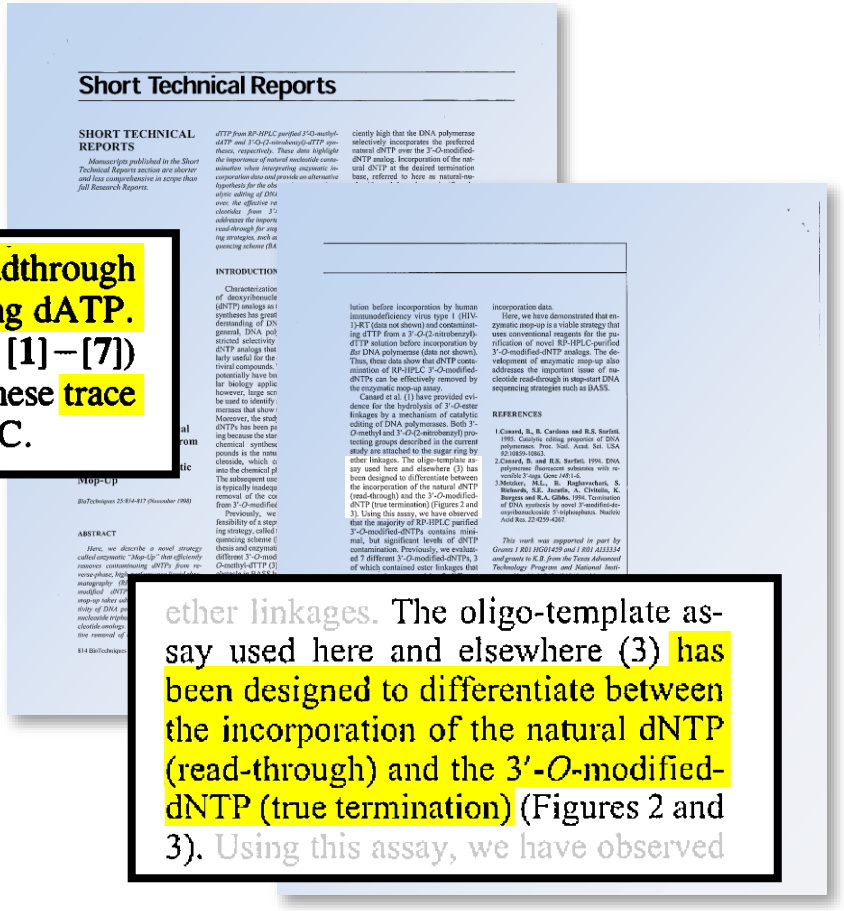
3′-*O*-Methyl-dATP [1] was also incorporated by M-MuLV-RT and inhibited DNA syntheses by *r*Tth and Vent(exo⁻)

Table 2. Activity matrix of RP-HPLC purified 3′-protecting dNTPs challenged against commercially available polymerases

3′-modified-dATP (except compound [8])	AMV-RT	M-MuLV-RT	Klenow fragment	Sequenase®	*Bst* DNA polymerase	AmpliTaq® DNA polymerase	Vent(exo⁻)® DNA polymerase	*r*Tth DNA polymerase
[1] O-methyl	Termination	Termination*	-	-	-	-	Inhibition	Inhibition*
[2] O-acyl	-	-	-	-	-	-	Inhibition	-
[3] O-allyl	-	-	-	-	-	-	Termination*	-
[4] O-benzhydroxyamn	-	-	-	-	-	-	-	-
[5] O-(4-nitrobenzoyl)	-	-	-	-	-	-	-	-
[6] O-(2-aminobenzoyl)	-	-	-	-	-	-	-	-
[7] O-(2-nitrobenzyl)	-	-	-	Inhibition	Termination	Termination*	Termination*	-
[8] 3′-O-methyl-dTTP	-	Inhibition	-	Inhibition	Termination	Termination	Termination	Termination

All compounds were assayed at a final concentration of 250 μM according to the conditions specified in Table 1. '–' means no activity was detected. 'Termination' means that the termination bands mimic dNTP termination bands, and 'Inhibition' means the rate of DNA synthesis is reduced in a nonspecific manner. '*' means the activity was incomplete at a final concentration of 250 μM .

Ex. 1016, Metzker (1994), 4263



Short Technical Reports

SHORT TECHNICAL REPORTS

Manuscripts published in the Short Technical Reports section are shorter and less comprehensive in scope than full Research Reports.

dTTP from RP-HPLC purified 3′-O-methyl-dATP and 3′-O-(2-nitrobenzyl)-dTTP syntheses, respectively. These data highlight the importance of natural nucleotide contamination when interpreting enzymatic incorporation data and provide an alternative hypothesis for the obs… ymatic editing of DNA … over, the effective re… chemistks from 3′-… addresses the importa… read-through for seq… ng strategies, such as … quencing scheme (BA…

ciently high that the DNA polymerase selectively incorporates the preferred natural dNTP over the 3′-*O*-modified-dNTP analog. Incorporation of the natural dNTP at the desired termination base, referred to here as natural-tu-

INTRODUCTION

Characterization of deoxyribonucle… (dNTP) analogs as … syntheses has great… dermasking of DN… general, DNA pol… stricted selectivity … dNTP analogs that … larly useful for the … tiviral compounds. … potentially have bro… lar biology applic… however, large scr… be used to identify … mechanics that show … Moreover, the study … dNTPs has been pa… ing because the star… chemical synthese… pounds is the name … cleoside, which … into the chemical pl… The subsequent use … is typically indepe… removal of the coo… from 3′-*O*-modified …

Previously, we … feasibility of a step… ing strategy, called … quenching scheme (… thesis and enzymati… different 3′-*O*-mod… *O*-methyl-dTTP (3) …

Mop-Up

BioTechniques 15:814-817 (November 1993)

ABSTRACT

Here, we describe a novel strategy called enzymatic "Mop-Up" that efficiently removes contaminating dNTPs from re… verse-phase, high… matography (HP… modified dNTP… mop-up takes adv… tivity of DNA … nucleotide triph… cleotide-analog… tive removal of …

814 BioTechniques

lution before incorporation by human immunodeficiency virus type 1 (HIV-1)-RT (data not shown) and contaminating dTTP from a 3′-*O*-(2-nitrobenzyl)-dTTP solution before incorporation by *Bst* DNA polymerase (data not shown). Thus, these data show that dNTP contamination of RP-HPLC 3′-*O*-modified-dNTPs can be effectively removed by the enzymatic mop-up assay.

Canard et al. (1) have provided evidence for the hydrolysis of 3′-*O*-ester linkages by a mechanism of catalytic editing of DNA polymerases. Both 3′-*O*-methyl and 3′-*O*-(2-nitrobenzyl) protecting groups described in the current study are attached to the sugar ring by ether linkages. The oligo-template assay used here and elsewhere (3) has been designed to differentiate between the incorporation of the natural dNTP (read-through) and the 3′-*O*-modified-dNTP (true termination) (Figures 2 and 3). Using this assay, we have observed that the majority of RP-HPLC purified 3′-*O*-modified-dNTPs contains minimal, but significant levels of dNTP contamination. Previously, we evaluated 7 different 3′-*O*-modified-dNTPs, 3 of which contained ester linkages that

incorporation data.

Here, we have demonstrated that enzymatic mop-up is a viable strategy that uses conventional reagents for the purification of novel RP-HPLC-purified 3′-*O*-modified-dNTP analogs. The development of enzymatic mop-up also addresses the important issue of nucleotide read-through in stop-start DNA sequencing strategies such as BASS.

REFERENCES

1.Canard, B., B. Cardona and R.S. Sarfati. 1993. Catalytic editing properties of DNA polymerases. Proc. Natl. Acad. Sci. USA 92:10859-10863.
2.Canard, B. and R.S. Sarfati. 1994. DNA polymerase fluorescent substrates with reversible 3′-tags. Gene 148:1-6.
3.Metzker, M.L., R. Raghavachari, S. Richards, S.E. Jacutin, A. Civitello, K. Burgess and R.A. Gibbs. 1994. Termination of DNA synthesis by novel 3′-modified-deoxyribonucleoside 5′-triphosphates. Nucleic Acids Res. 22:4259-4267.

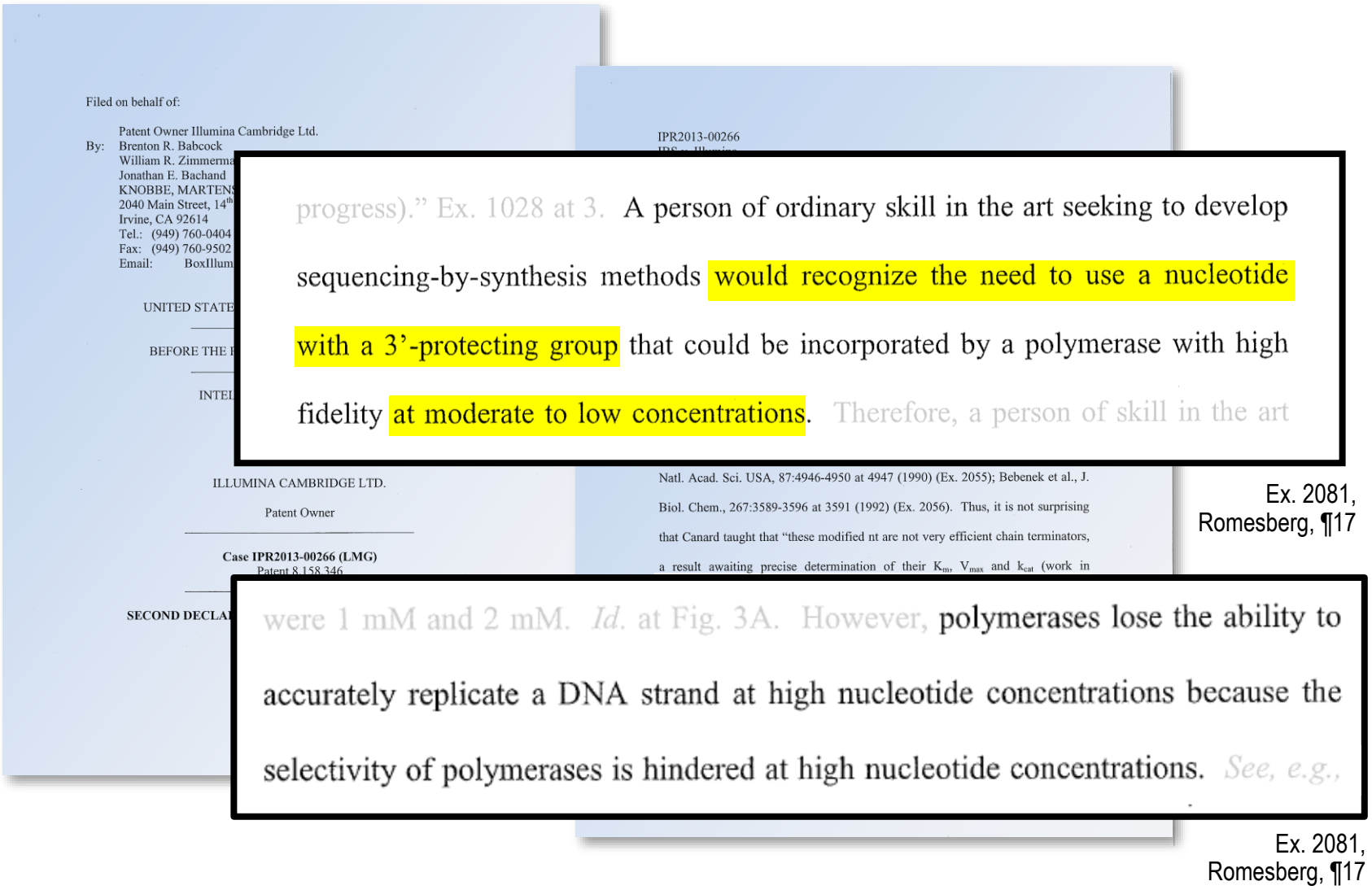
This work was supported in part by Grants 1 R01 HG01459 and 1 R01 AI33334 and grants to K.B. from the Texas Advanced Technology Program and National Insti…

ether linkages. The oligo-template assay used here and elsewhere (3) has been designed to differentiate between the incorporation of the natural dNTP (read-through) and the 3′-*O*-modified-dNTP (true termination) (Figures 2 and 3). Using this assay, we have observed

Ex. 2131, Metzker (1998), 817

IPR2018-00291, PO Response, 24; Sur-Reply, 10; Opp. Mot. Exclude, 4-5, 7-8

High Nucleotide Concentrations Are Incompatible With SBS

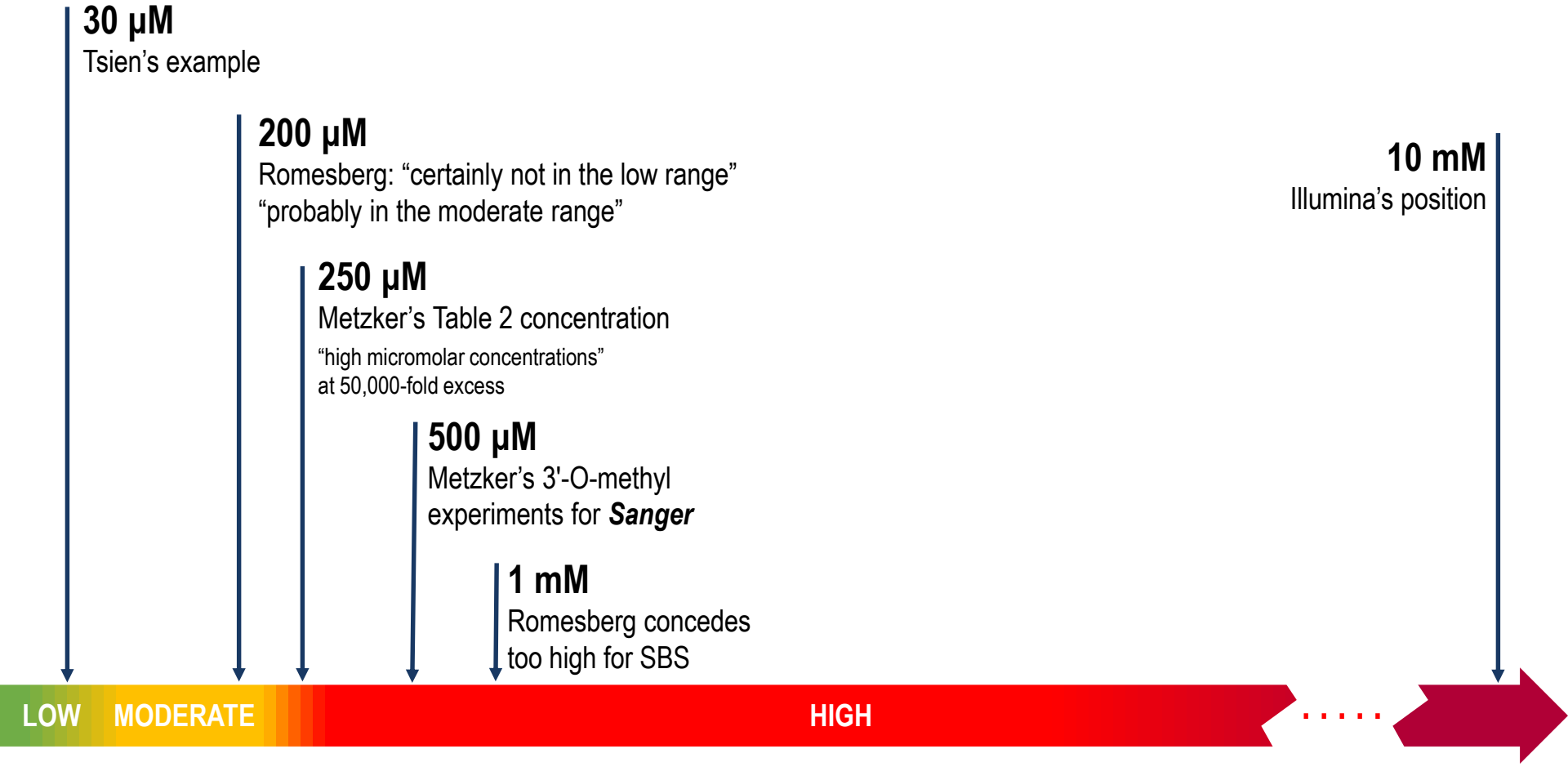


Ex. 2081, Romesberg, ¶17

Ex. 2081, Romesberg, ¶17

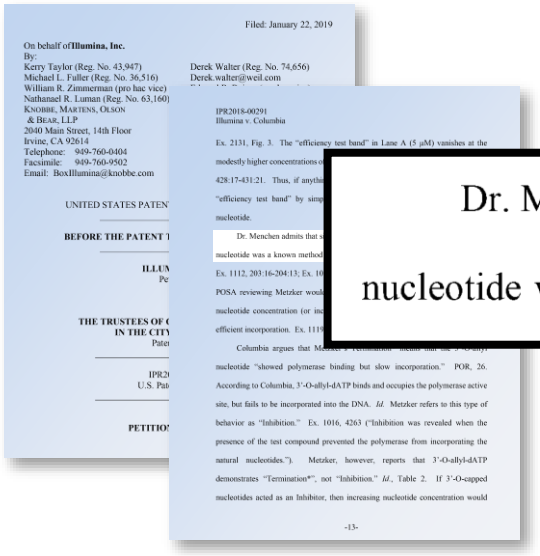
IPR2018-00291, Sur-Reply, 13-14; Opp. Mot. Exclude, 12-14

High Nucleotide Concentrations Are Incompatible With SBS



IPR2018-00291, Sur-Reply, 13-16; Opp. Mot. Exclude, 12-14

Dr. Menchen Did Not Testify That Increasing Concentration Increases Incorporation



IPR2018-00291
Illumina v. Columbia

Ex. 2131, Fig. 3. The "efficiency test band" in Lane A (5 μM) vanishes at the

modestly higher concentrations of

428:17-431:21. Thus, if anythi

"efficiency test band" by simp

nucleotide.

Dr. Menchen admits that s

nucleotide was a known method

Ex. 1112, 203:16-204:13; Ex. 10

POSA reviewing Metzker would

nucleotide concentration (or in

efficient incorporation. Ex. 1119

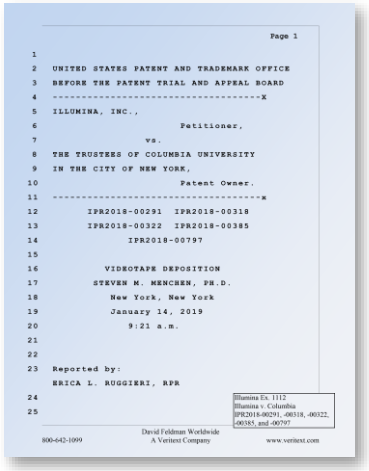
Columbia argues that M

nucleotide "showed polymerase binding but slow incorporation." POR, 26. According to Columbia, 3'-O-allyl-dATP binds and occupies the polymerase active site, but fails to be incorporated into the DNA. *Id.* Metzker refers to this type of behavior as "Inhibition." Ex. 1016, 4263 ("Inhibition was revealed when the presence of the test compound prevented the polymerase from incorporating the natural nucleotides."). Metzker, however, reports that 3'-O-allyl-dATP demonstrates "Termination*", not "Inhibition." *Id.*, Table 2. If 3'-O-capped nucleotides acted as an Inhibitor, then increasing nucleotide concentration would

-13-

> Dr. Menchen admits that simply increasing the concentration of 3'-O-capped nucleotide was a known method for achieving efficient polymerase incorporation.

IPR2018-00291, Reply, 13



Page 1

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,
vs.
THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK,
Patent Owner.

IPR2018-00291 IPR2018-00318
IPR2018-00322 IPR2018-00385
IPR2018-00797

VIDEOTAPE DEPOSITION
STEVEN M. MENCHEN, PH.D.
New York, New York
January 14, 2019
9:21 a.m.

Reported by:
ERICA L. RUGGIERI, RPR

Illumina Ex. 1112
Illumina v. Columbia
IPR2018-00291, -00318, -00322, -00385, and -00797

800-642-1099 David Feldman Worldwide A Veritext Company www.veritext.com

Stephen Menchen, Ph.D.
Deposition

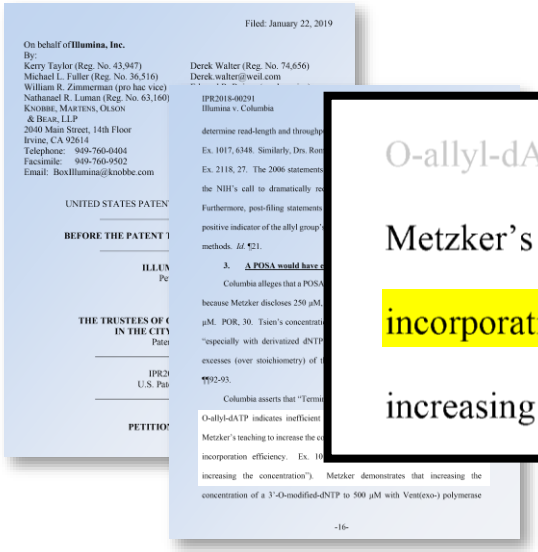
Q. Based on the, your expertise in the Metzker paper, is it your belief that increasing the concentration increases efficiency until you are basically a hundred percent efficient?

A. No. I -- I don't think you can assume that.

Ex. 1112, Menchen Dep., 206:23-207:6

IPR2018-00291, Sur-Reply, 15-16

Metzker 1994 Does Not Teach That Increasing Concentration Solves Problem of Inefficient Incorporation



O-allyl-dATP indicates inefficient incorporation. POR, 21. Columbia ignores Metzker's teaching to increase the concentration of 3'-O-modified-dNTP to enhance incorporation efficiency. Ex. 1016, 4266 ("problems could be resolved by increasing the concentration"). Metzker demonstrates that increasing the

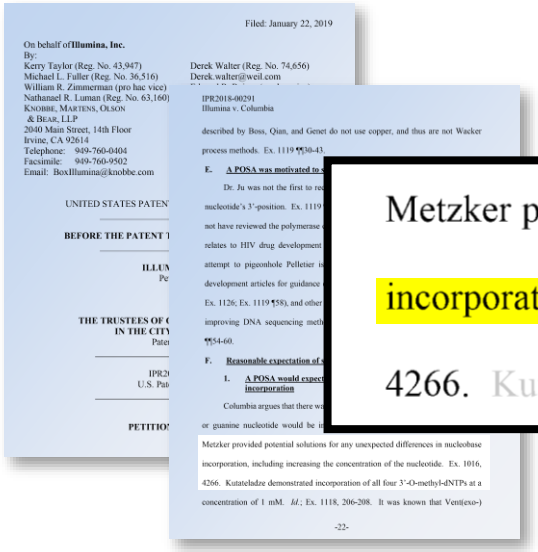
IPR2018-00291, Reply, 16

Several unexpected results were observed in the use of the 3′-*O*-methyl-dNTPs as terminators in DNA sequencing. First, the 3′-*O*-methyl-dTTP [8] reduced enzyme fidelity. Also, we have not identified a polymerase that will incorporate both 3′-*O*-methyl-dATP [1] and 3′-*O*-methyl-dTTP [8]. These problems could be resolved by increasing the concentration of dATP in the former case to titrate away artifact termination bands and by testing more polymerases in the latter case. This issue, however,

Ex. 1016, Metzker (1994), 4266

IPR2018-00291, Sur-Reply, 14-15

Metzker 1994 Does Not Teach That Increasing Concentration Solves Differences in Nucleobase Incorporation



Filed: January 22, 2019

On behalf of **Illumina, Inc.**

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UNITED STATES PATEN

BEFORE THE PATENT

THE TRUSTEES OF
IN THE CITY

PETITIO

IPR2018-00291
Illumina v. Columbia

described by Boss, Qian, and Genet do not use copper, and thus are not Wacker

process methods. Ex. 1119 ¶¶30-43.

E. **A POSA was motivated to**

Dr. Ju was not the first to re

nucleotide's 3'-position. Ex. 1119

not have reviewed the polymerase

relates to HIV drug development

attempt to pigeonhole Pelletier i

development articles for guidance

Ex. 1126; Ex. 1119 ¶58), and other

improving DNA sequencing meth

¶¶54-60.

F. **Reasonable expectation of**

1. **A POSA would expect incorporation**

Columbia argues that there wo

or guanine nucleotide would be i

Metzker provided potential solutions for any unexpected differences in nucleobase incorporation, including increasing the concentration of the nucleotide. Ex. 1016, 4266. Kutateladze demonstrated incorporation of all four 3'-O-methyl-dNTPs at a concentration of 1 mM. *Id.*; Ex. 1118, 206-208. It was known that Vent(exo-)

-22-

> Metzker provided potential solutions for any unexpected differences in nucleobase incorporation, including increasing the concentration of the nucleotide. Ex. 1016, 4266. Kutateladze demonstrated incorporation of all four 3'-O-methyl-dNTPs at a

IPR2018-00291, Reply, 22

© 1994 Oxford University Press — *Nucleic Acids Research, 1994, Vol. 22, No. 20 4259–4267*

Termination of DNA synthesis by novel 3'-modified-deoxyribonucleoside 5'-triphosphates

Michael L.Metzker*, Ramesh Raghavachari[+], Stephen Richards, Swanee E.Jacutin[+], Andrew Civitello, Kevin Burgess[+] and Richar

Department of Molecular and Human Genetics, Houston, TX 77030 and [+]Department of Chem TX 77843, USA

Received June 3, 1994; Revised and Accepted July 2

ABSTRACT

INTRODUCTION

*To whom correspondence should be addressed

[+]Present address: LI-COR Inc., Biotechnology Division, Lincoln, NE

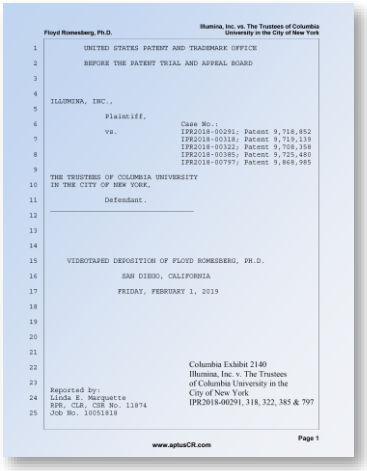
Nucleic Acids Research, 1994, Vol. 22, No. 20 4266

Several unexpected results were observed in the use of the 3′-*O*-methyl-dNTPs as terminators in DNA sequencing. First, the 3′-*O*-methyl-dTTP [8] reduced enzyme fidelity. Also, we have not identified a polymerase that will incorporate both 3′-*O*-methyl-dATP [1] and 3′-*O*-methyl-dTTP [8]. These problems could be resolved by increasing the concentration of dATP in the former case to titrate away artifact termination bands and by testing more polymerases in the latter case. This issue, however,

Ex. 1016, Metzker (1994), 4266

IPR2018-00291, Sur-Reply, 25

Vent Variants: Illumina Provides No Evidence That a POSA Would Expect Vent Variants to Efficiently Incorporate 3'-O-Allyl-dATP


Floyd Romesberg, Ph.D. — Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,

Plaintiff,

vs.

Case No.:
IPR2018-00291; Patent 9,718,852
IPR2018-00318; Patent 9,719,139
IPR2018-00322; Patent 9,708,358
IPR2018-00385; Patent 9,725,480
IPR2018-00797; Patent 9,868,985

THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,

Defendant.

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.

SAN DIEGO, CALIFORNIA

FRIDAY, FEBRUARY 1, 2019

Columbia Exhibit 2140
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291, 318, 322, 385 & 797

Reported by:
Linda E. Marquette
RPR, CLR, CSR No. 11874
Job No. 10051810

www.aptusCR.com

Page 1

Floyd Romesberg, Ph.D.
Deposition

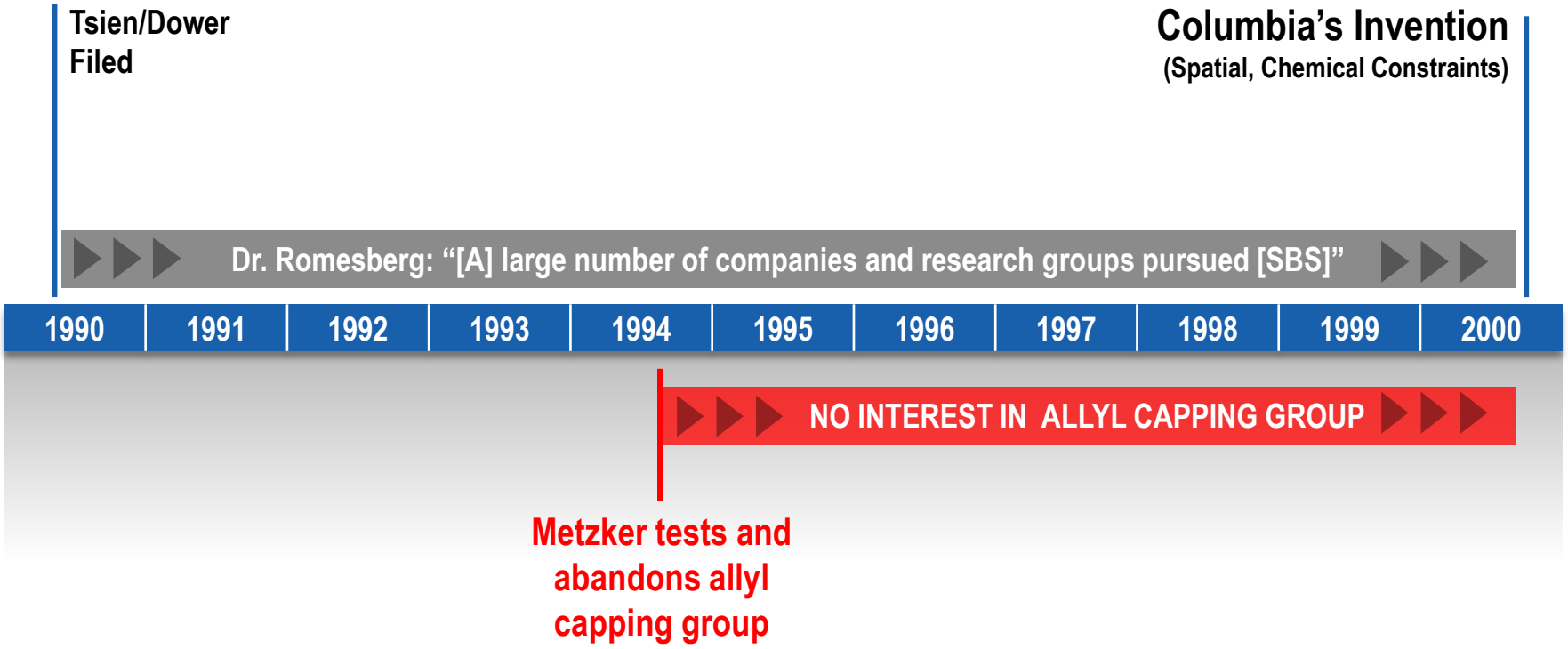
Q. And there's no experimental data in Gardner that demonstrates efficient incorporation of a 3'-O-propenyl modified nucleotide with mutated vent (exo-) polymerase, correct?

A. No. What I was suggesting this as is that a POSA would have known that there were mutants of vent that were more tolerant to the 3' position. He would not have known the[y] accepted 3'-O-allyl. My only point in the dec, to be clear, was that a POSA would have known to go look. He would -- he would not have known. He would have known to look and try.

Ex. 2140, Romesberg Dep., 130:12-22

IPR2018-00291, Sur-Reply, 16; Opp. Mot. Exclude, 8, 14

Illumina's Theories Are Belied by Real-World Evidence



IPR2018-00291, PO Response, 23, 27; Sur-Reply, 2, 5, 9-10, 16

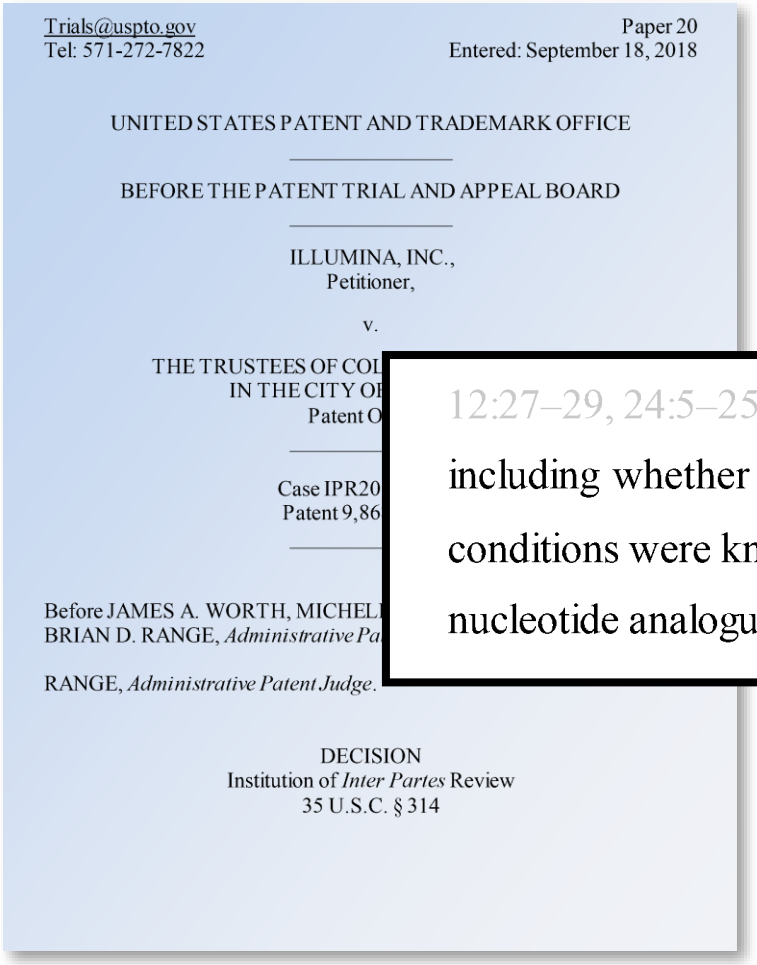
Illumina's Cleavage Condition Arguments Fail

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

The Board Requested Briefing Regarding Allyl Ether Cleavage Conditions



Trials@uspto.gov
Tel: 571-272-7822

Paper 20
Entered: September 18, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

v.

THE TRUSTEES OF COL
IN THE CITY OF
Patent O

Case IPR20
Patent 9,86

Before JAMES A. WORTH, MICHEL
BRIAN D. RANGE, *Administrative Pa*

RANGE, *Administrative Patent Judge*.

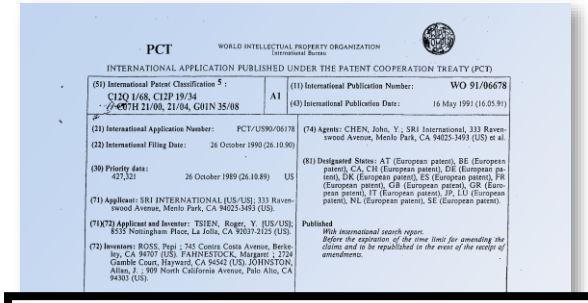
DECISION
Institution of *Inter Partes* Review
35 U.S.C. § 314

12:27–29, 24:5–25:3. We invite further briefing on this issue at trial, including whether it was appreciated in the art that sufficiently mild conditions were known for cleaving allyl groups when working with nucleotide analogues. We also invite briefing on (1) whether or not the

IPR2018-00797, Institution Decision, 25-26

IPR2018-00291, PO Response, 2, 31

No Dispute SBS (BASS) Requires SBS-Compatible Cleavage Conditions



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau

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(74) Agents: CHEN, John, Y.; SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025-3493 (US) et al.

(81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European patent), DK (European patent), ES (European patent), FR (European patent), GB (European patent), GR (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent).

Published

With international search report.

Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

Blocking Groups and Methods for Incorporation

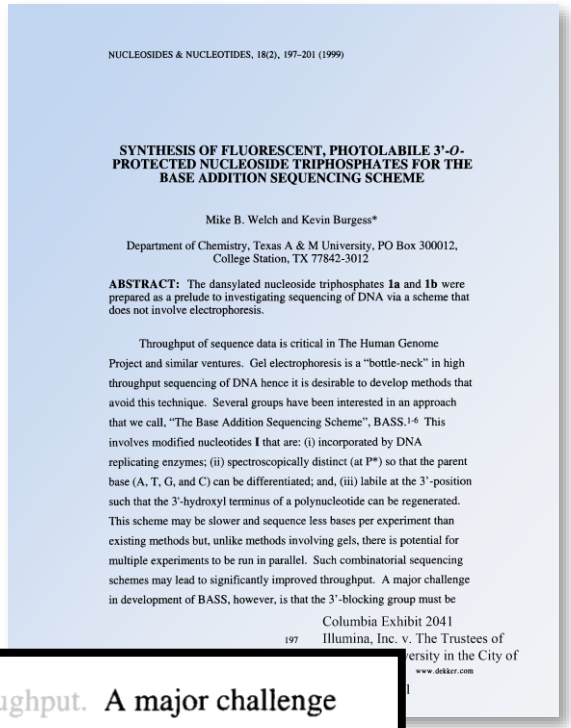
The coupling reaction generally employs 3'hydroxyl-blocked dNTPs to prevent inadvertent extra additions.

The criteria for the successful use of 3'-blocking groups include:

(1) the ability of a polymerase enzyme to accurately and efficiently incorporate the dNTPs carrying the 3'-blocking groups into the cDNA chain,

(2) the availability of mild conditions for rapid and quantitative deblocking, and

Ex. 1013, Tsien, 20



NUCLEOSIDES & NUCLEOTIDES, 18(2), 197–201 (1999)

SYNTHESIS OF FLUORESCENT, PHOTOLABILE 3'-*O*-PROTECTED NUCLEOSIDE TRIPHOSPHATES FOR THE BASE ADDITION SEQUENCING SCHEME

Mike B. Welch and Kevin Burgess*

Department of Chemistry, Texas A & M University, PO Box 300012, College Station, TX 77842-3012

ABSTRACT: The dansylated nucleoside triphosphates **1a** and **1b** were prepared as a prelude to investigating sequencing of DNA via a scheme that does not involve electrophoresis.

Throughput of sequence data is critical in The Human Genome Project and similar ventures. Gel electrophoresis is a "bottle-neck" in high throughput sequencing of DNA hence it is desirable to develop methods that avoid this technique. Several groups have been interested in an approach that we call, "The Base Addition Sequencing Scheme", BASS.[1-6] This involves modified nucleotides **I** that are: (i) incorporated by DNA replicating enzymes; (ii) spectroscopically distinct (at P*) so that the parent base (A, T, G, and C) can be differentiated; and, (iii) labile at the 3'-position such that the 3'-hydroxyl terminus of a polynucleotide can be regenerated. This scheme may be slower and sequence less bases per experiment than existing methods but, unlike methods involving gels, there is potential for multiple experiments to be run in parallel. Such combinatorial sequencing schemes may lead to significantly improved throughput. A major challenge in development of BASS, however, is that the 3'-blocking group must be

Columbia Exhibit 2041
Illumina, Inc. v. The Trustees of
...versity in the City of

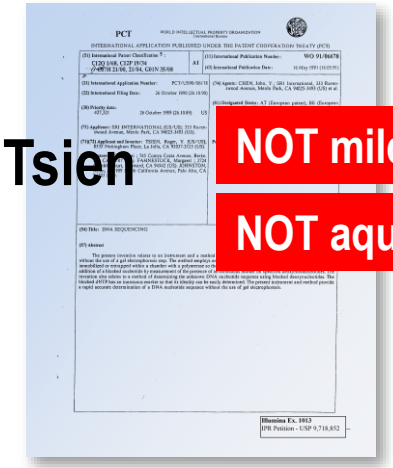
schemes may lead to significantly improved throughput. A major challenge in development of BASS, however, is that the 3'-blocking group must be removed in an aqueous medium using reagents/conditions that do not denature or modify double strand DNA. To address this issue, the focus of

Ex. 2041, Welch, 197-98

IPR2018-00291, PO Response, 6, 31

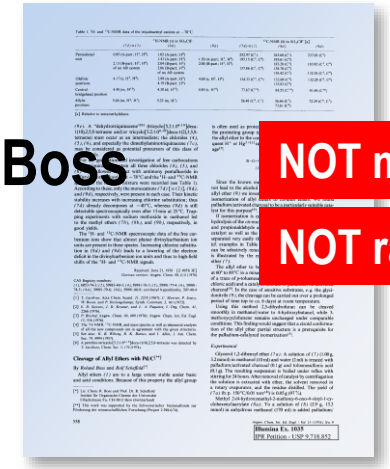
Illumina's Petition-Cited References Do Not Teach SBS-Compatible Cleavage Conditions

Tsien



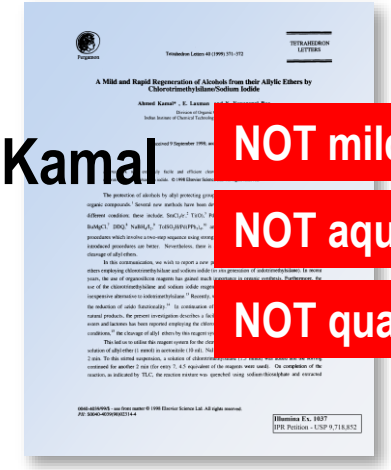
- NOT mild
- NOT aqueous

Boss



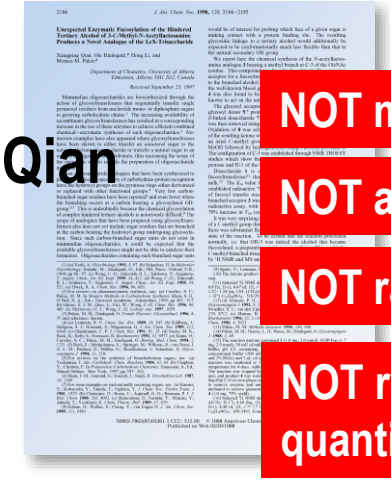
- NOT mild
- NOT rapid

Kamal



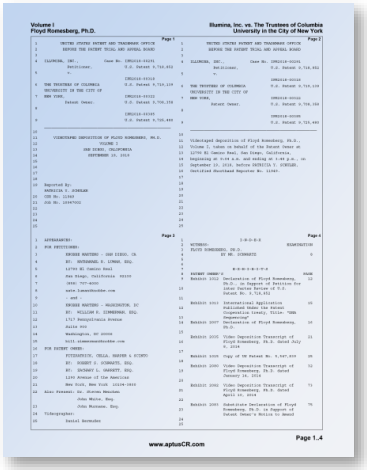
- NOT mild
- NOT aqueous
- NOT quantitative

Qian



- NOT mild
- NOT aqueous
- NOT rapid
- NOT reliably quantitative

Tsien Does Not Teach SBS-Compatible Cleavage Conditions



Floyd Romesberg, Ph.D.
Deposition

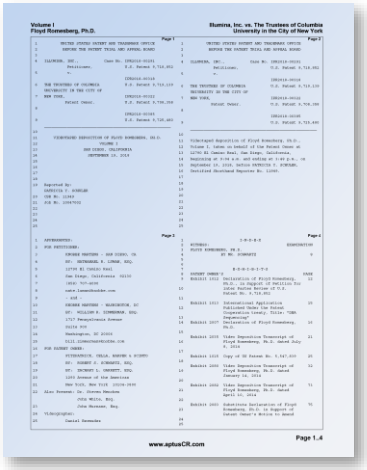
Q. So a person reading Tsien along with Gigg and Warren would not use Gigg's condition for cleavage of allyl ethers for SBS because there would be no expectation of success using those cleavage conditions, correct?

A. I think that is fair.

Ex. 2126, Romesberg Dep., 232:10-15

IPR2018-00291, PO Response, 33

Kamal Does Not Teach SBS-Compatible Cleavage Conditions



Floyd Romesberg, Ph.D.
Deposition

Q. But [Kamal's] reaction takes place in acetonitrile with no water present?

A. Yes. And in that case it would denature the DNA. And you would dehydrate the DNA and cause it to denature.

Q. And Kamal's allyl deprotection method uses chlorotrimethylsilane, correct?

A. It does.

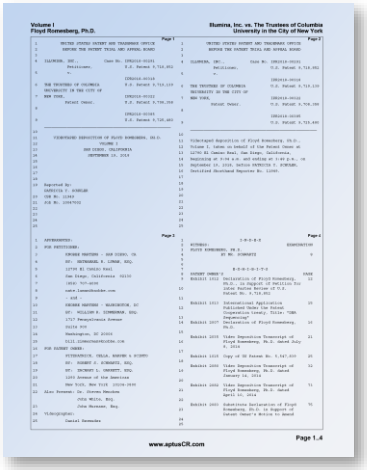
Q. And chlorotrimethylsilane is not compatible with water, correct?

A. No, it is not.

Ex. 2126, Romesberg Dep., 234:23-235:8

IPR2018-00291, PO Response, 35

Boss Does Not Teach SBS-Compatible Cleavage Conditions



Floyd Romesberg, Ph.D.
Deposition

Q. So the concentration of perchloric acid that was used to deprotect the allyl in the Boss deprotection reaction would denature DNA, correct?

A. The Kit reference suggests that it would.

Q. And that deprotection reaction in Boss would not be considered a mild condition that would not denature DNA, correct?

A. That is correct.

* * *

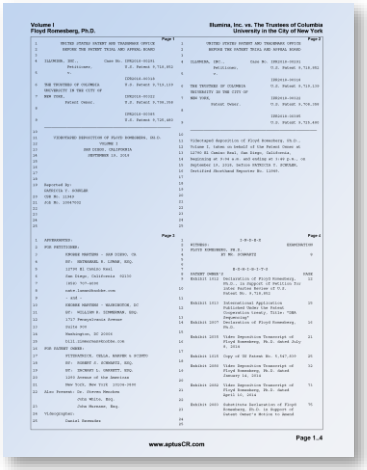
Q. So none of Boss's reaction conditions satisfied Tsien's requirement for rapid deprotection, correct?

A. I would agree with that.

Ex. 2126, Romesberg Dep., 190:7-14, 203:17-20

IPR2018-00291, PO Response, 37-39

Qian Does Not Teach SBS-Compatible Cleavage Conditions



Floyd Romesberg, Ph.D.
Deposition

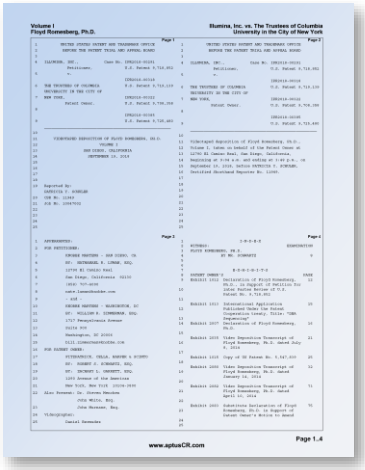
Q. But this reference that you are relying on [Qian] to say that there was quantitative cleavage of the allyl protecting group uses conditions that are not themselves compatible with SBS, correct?

A. That is correct.

Ex. 2126, Romesberg Dep., 163:23-164:2

IPR2018-00291, PO Response, 40

Dr. Romesberg Concedes if Tsien's Cleavage Conditions Are Not Met, It Will Not Work for Tsien's SBS Methods



Floyd Romesberg, Ph.D.
Deposition

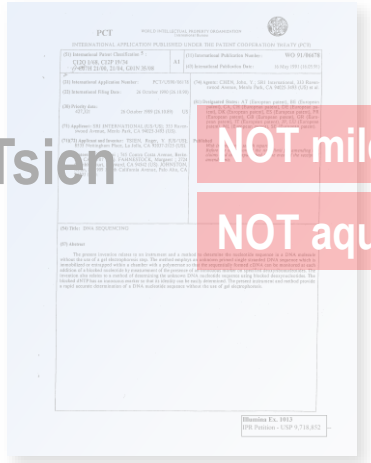
Q. So according to Tsien, if any of these criteria are not met, Tsien says it wouldn't be expected to work for his SBS method, correct?

A. Yes.

Ex. 2126, Romesberg Dep., 45:1-4

IPR2018-00291, PO Response, 3-6

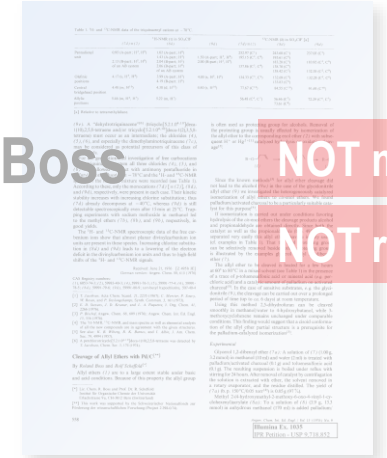
Illumina's New Qian Argument



Tsien

NOT mild

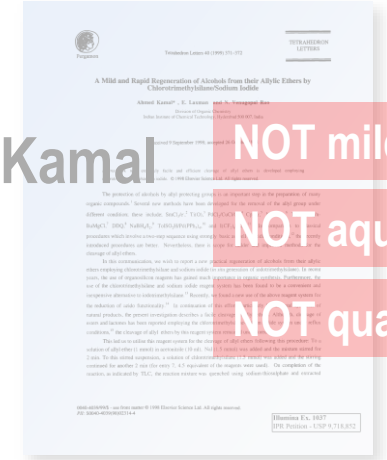
NOT aqueous



Boss

NOT mild

NOT rapid



Kamal

NOT mild

NOT aqueous

NOT quantitative

Qian

NOT mild

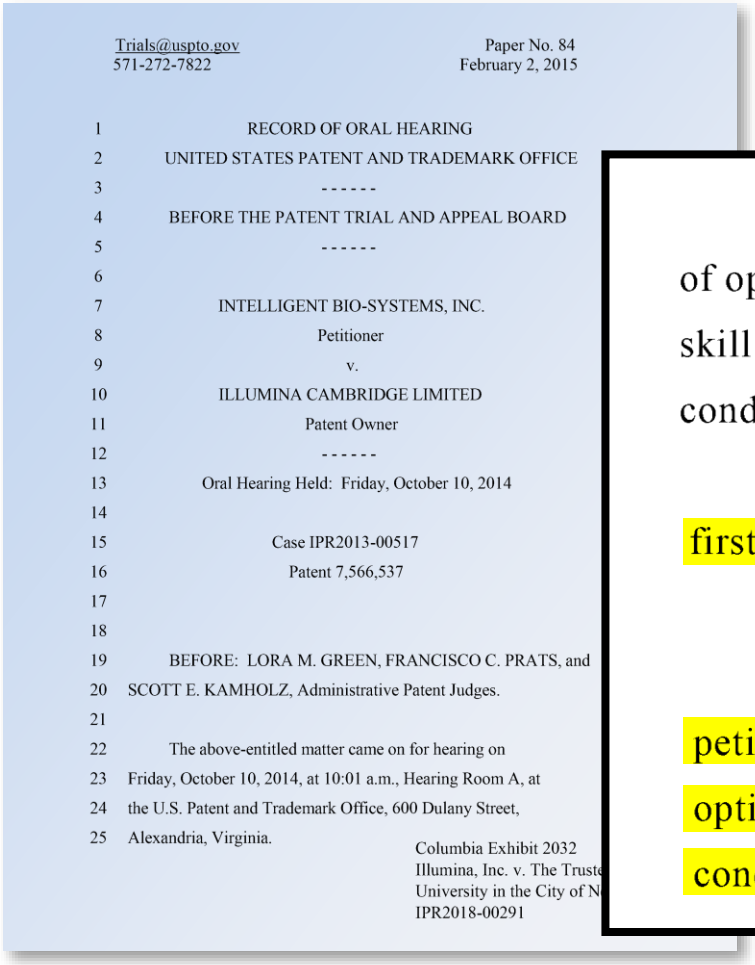
NOT aqueous

NOT rapid

Illumina belatedly argues Qian could be modified to be aqueous using 3 Genet references, which are not for allyl ether cleavage

IPR2018-00291, Sur-Reply, 18-19

Illumina Previously Argued Against Allowing Newly Proposed Cleavage Conditions In Reply Briefing



Trials@uspto.gov
571-272-7822

Paper No. 84
February 2, 2015

RECORD OF ORAL HEARING
UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

INTELLIGENT BIO-SYSTEMS, INC.
Petitioner
v.
ILLUMINA CAMBRIDGE LIMITED
Patent Owner

Oral Hearing Held: Friday, October 10, 2014

Case IPR2013-00517
Patent 7,566,537

BEFORE: LORA M. GREEN, FRANCISCO C. PRATS, and SCOTT E. KAMHOLZ, Administrative Patent Judges.

The above-entitled matter came on for hearing on Friday, October 10, 2014, at 10:01 a.m., Hearing Room A, at the U.S. Patent and Trademark Office, 600 Dulany Street, Alexandria, Virginia.

Columbia Exhibit 2032
Illumina, Inc. v. The Truste
University in the City of N
IPR2018-00291

illumina

Now, I would like to spend some time on the issue of optimization, because you heard here today that one of skill in the art could easily change Zavgorodny's removal conditions and optimize.

This is an argument that came out on reply for the first time. It is an improper new prima facie case. If you

* * *

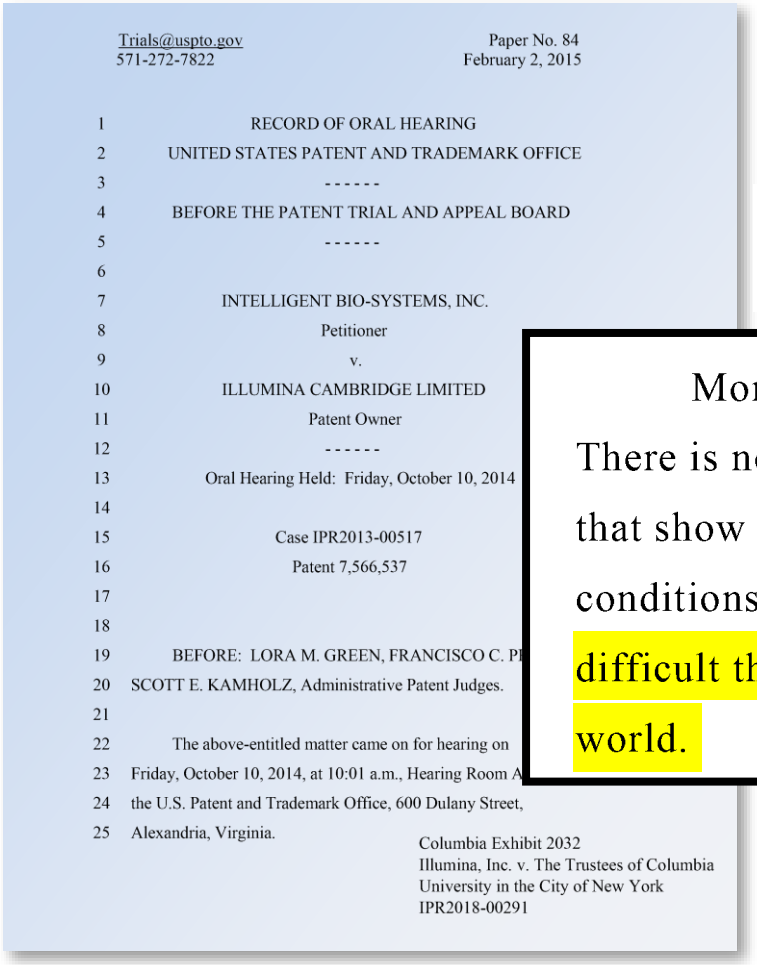
And so they are limited to the conditions in the petition. The petition didn't say one word about optimization. It didn't say one word about changing the conditions. This is a completely new argument on reply.

Ex. 2032, Illumina, Oral Hearing Tr., 46:5-10, 20-23

Intelligent Bio-Systems, Inc. v. Illumina Cambridge Ltd., 821 F.3d 1359, 1369 (Fed. Cir. 2016) (Federal Circuit agreeing)

IPR2018-00291, Sur-Reply, 18

Illumina Previously Argued That Modifying Cleavage Conditions Is Difficult



Trials@uspto.gov
571-272-7822

Paper No. 84
February 2, 2015

RECORD OF ORAL HEARING
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the U.S. Patent and Trademark Office, 600 Dulany Street,
Alexandria, Virginia.

Columbia Exhibit 2032
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291

illumina

Moreover, IBS didn't give you any test data. There is no test results that says, oh, here are the tests that show you can very easily optimize Zavgorodny's removal conditions. They didn't present them because they know how difficult that is. This just isn't what happens in the real world.

Ex. 2032, Illumina, Oral Hearing Tr., 48:10-15

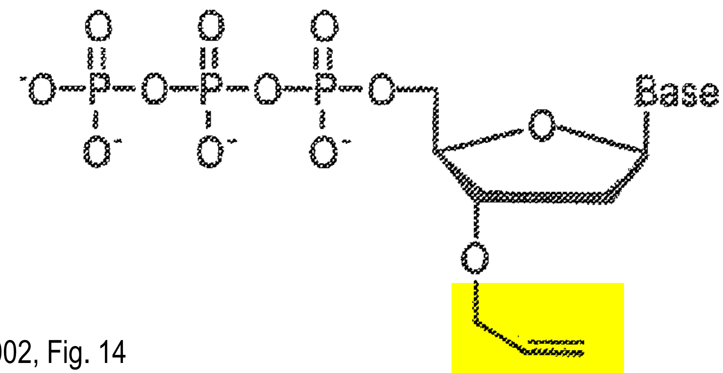
IPR2018-00291, Sur-Reply, 4-5, 18-19

Illumina's New References Relate to Alloc, Which Is a Carbonate Protecting Group, Not an Ether

Columbia's Patent


United States Patent
Ju et al.
Patent No.: US 9,708,358 B2
Date of Patent: *Jul. 18, 2017

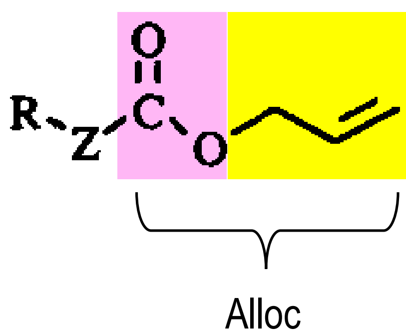
Columbia Capping Group: An Allyl ***Ether***



Ex. 1002, Fig. 14

Lemaire-Audoire, et al.

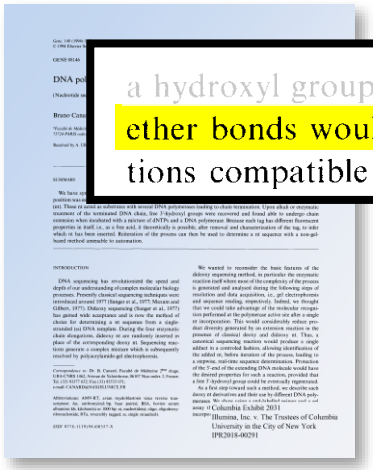
Illumina's Belated References: Allyl ***Oxy Carbonyl*** ("Alloc")



Ex. 1115, 248

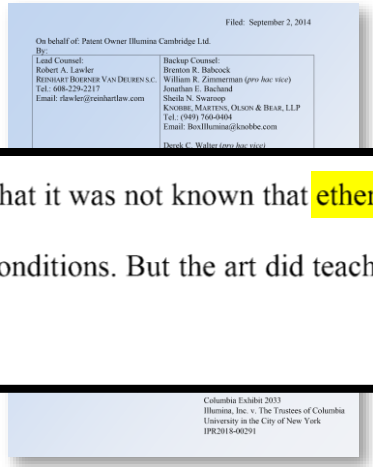
IPR2018-00291, Sur-Reply, 18-19

Illumina Concedes That the Prior Art Taught That Ethers Are Hard to Cleave



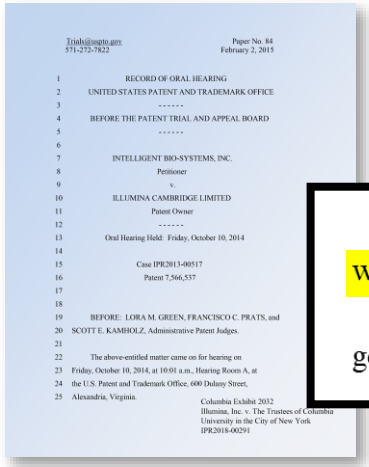
a hydroxyl group upon deprotection. We reasonned that ether bonds would be hard to cleave under mild conditions compatible with DNA chemical stability, whereas

Ex. 2031, Canard, 4



7. In Ex. 2154 at 81:8-13, Dr. Metzker testified that it was not known that ether bonds were hard to cleave under DNA compatible conditions. But the art did teach this. Ex. 2043, p. 4; Ex. 1002, 3:9-14.

Ex. 2033, Illumina, 3



illumina

Canard's '94 paper, Exhibit 2043, expressly states: We reasoned that ether bonds would be hard to cleave under mild conditions compatible with DNA chemical stability.

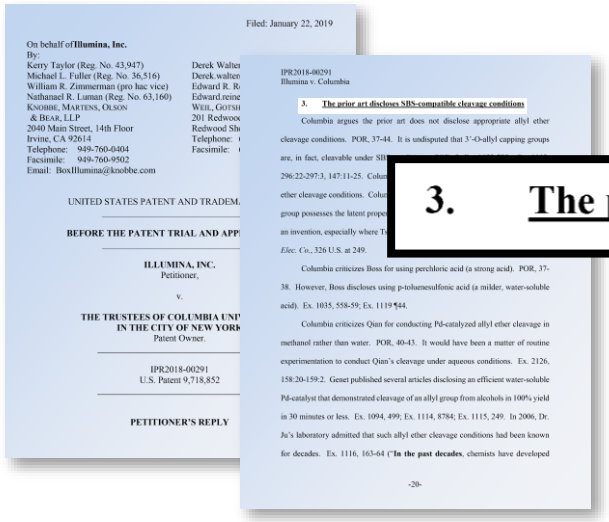
So even in '94 there was a hint that the conditions needed to cleave an ether weren't going to work in DNA synthesis.

Ex. 2032, Illumina, Oral Hearing Tr., 37:7-12

Illumina failed to provide evidence that alloc references teach allyl ether cleavage under SBS-compatible conditions

IPR2018-00291, Opp. Mot. Exclude, 15; Sur-Reply, 18-19

Illumina's Current Position That SBS-Compatible Allyl Ether Cleavage Conditions Were Known Prior to 2000 Contradicts Its Previous Position



Filed: January 22, 2019

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UNITED STATES PATENT AND TRADEM...

BEFORE THE PATENT TRIAL AND APP...

ILLUMINA, INC.
Petitioner,
v.
THE TRUSTEES OF COLUMBIA UNI... IN THE CITY OF NEW YORK
Patent Owner.

IPR2018-00291
U.S. Patent 9,718,852

PETITIONER'S REPLY

IPR2018-00291
Illumina v. Columbia

3. **The prior art discloses SBS-compatible cleavage conditions**

Columbia argues the prior art does not disclose appropriate allyl ether cleavage conditions. POR, 37-44. It is undisputed that 3'-O-allyl capping groups are, in fact, cleavable under SB... 296:22-297:3, 147:11-25. Colum... ether cleavage conditions. Colum... group possesses the latent prope... an invention, especially where T... *Elec. Co.*, 326 U.S. at 249.

Columbia criticizes Boss for using perchloric acid (a strong acid). POR, 37-38. However, Boss discloses using p-toluenesulfonic acid (a milder, water-soluble acid). Ex. 1035, 558-59; Ex. 1119 ¶44.

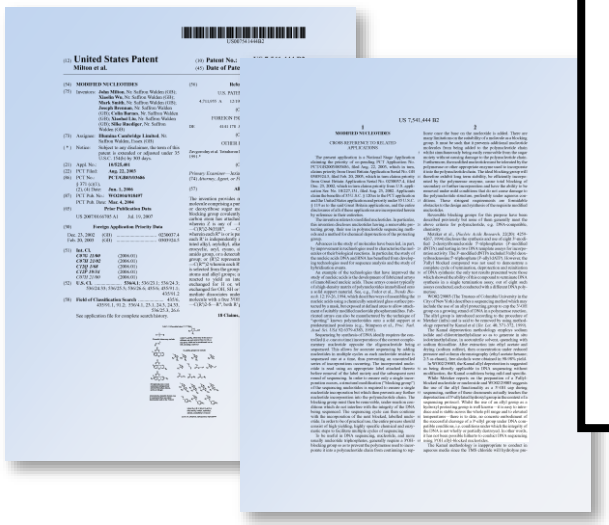
Columbia criticizes Qian for conducting Pd-catalyzed allyl ether cleavage in methanol rather than water. POR, 40-43. It would have been a matter of routine experimentation to conduct Qian's cleavage under aqueous conditions. Ex. 2126, 158:20-159:2. Genet published several articles disclosing an efficient water-soluble Pd-catalyst that demonstrated cleavage of an allyl group from alcohols in 100% yield in 30 minutes or less. Ex. 1094, 499; Ex. 1114, 8784; Ex. 1115, 249. In 2006, Dr. Ju's laboratory admitted that such allyl ether cleavage conditions had been known for decades. Ex. 1116, 163-64 ("**In the past decades**, chemists have developed

-20-

Illumina's Current Position

3. The prior art discloses SBS-compatible cleavage conditions

IPR2018-00291, Reply, 20



United States Patent
Milton et al.

US 7,541,444 B2

MODIFIED NUCLEOTIDES

Illumina's Previous Position

sequencing protocol. Whilst the use of an allyl group as a hydroxyl protecting group is well known—it is easy to introduce and is stable across the whole pH range and to elevated temperatures—there is to date, no concrete embodiment of the successful cleavage of a 3'-allyl group under DNA compatible conditions, i.e. conditions under which the integrity of the DNA is not wholly or partially destroyed. In other words, it has not been possible hitherto to conduct DNA sequencing using 3'OH allyl-blocked nucleotides.

Ex. 2015, '444 Illumina Patent, 2:57-65

IPR2018-00291, PO Response, 36-37; Sur-Reply, 27-28

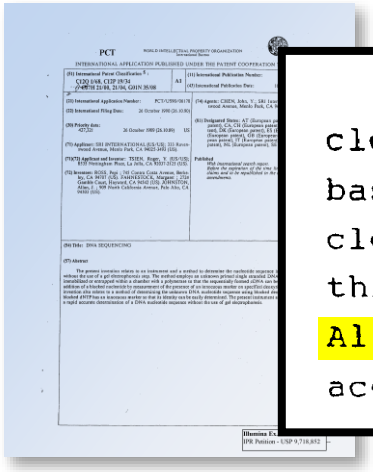
Illumina's Tsien-Based Arguments Fail

Relevant to:

IPR2018-00291, 318, 322, 385: Ground 1

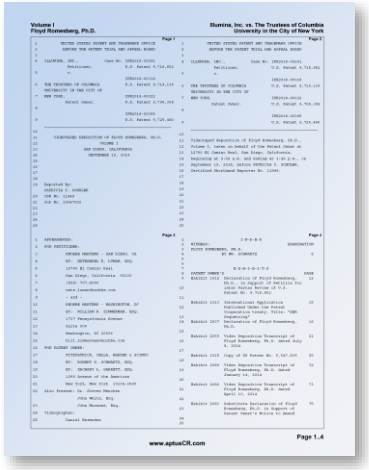
IPR2018-00797: Grounds 1-2

Tsien Does Not Describe the -CH_2-CH=CH_2 Capping Group



A wide variety of hydroxyl blocking groups are cleaved selectively using chemical procedures other than base hydrolysis. 2,4-Dinitrobenzenesulfenyl groups are cleaved rapidly by treatment with nucleophiles such as thiophenol and thiosulfate (Letsinger et al., 1964). Allyl ethers are cleaved by treatment with Hg(II) in acetone/water (Gigg and Warren, 1968).

Ex. 1013, Tsien, 24



Floyd Romesberg, Ph.D.
Deposition

Q. And throughout Gigg and Warren there are disclosures of allyl ethers that are both unsubstituted and substituted, correct?

A. Apparently, yes.

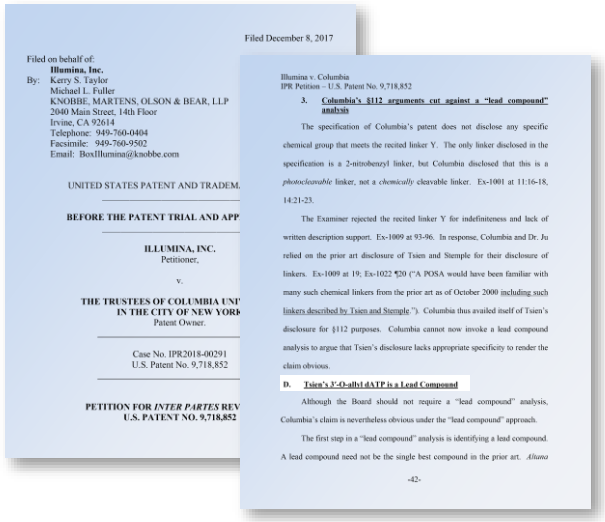
Q. And throughout Gigg and Warren there are also disclosures of allyl ethers other than a three carbon allyl ether, correct?

A. That is correct. Obviously, from what you just pointed out. At least.

Ex. 2126, Romesberg Dep., 214:5-13

IPR2018-00291, PO Response, 53-54; Sur-Reply, 20-22

Illumina's Current Position That Tsien Describes the -CH_2-CH=CH_2 Capping Group Contradicts Its Previous Position



Filed December 8, 2017

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UNITED STATES PATENT AND TRADEM...

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Case No. IPR2018-00291
U.S. Patent No. 9,718,852

PETITION FOR *INTER PARTES* REV...
U.S. PATENT NO. 9,718,852

Illumina v. Columbia
IPR Petition – U.S. Patent No. 9,718,852

3. **Columbia's §112 arguments cut against a "lead compound" analysis**

The specification of Columbia's patent does not disclose any specific chemical group that meets the recited linker Y. The only linker disclosed in the specification is a 2-nitrobenzyl linker, but Columbia disclosed that this is a *photocleavable* linker, not a *chemically* cleavable linker. Ex-1001 at 11:16-18, 14:21-23.

The Examiner rejected the recited linker Y for indefiniteness and lack of written description support. Ex-1009 at 93-96. In response, Columbia and Dr. Ju relied on the prior art disclosure of Tsien and Stemple for their disclosure of linkers. Ex-1009 at 19; Ex-1022 ¶20 ("A POSA would have been familiar with many such chemical linkers from the prior art as of October 2000 including such linkers described by Tsien and Stemple."). Columbia thus availed itself of Tsien's disclosure for §112 purposes. Columbia cannot now invoke a lead compound analysis to argue that Tsien's disclosure lacks appropriate specificity to render the claim obvious.

D. **Tsien's 3′-O-allyl dATP is a Lead Compound**

Although the Board should not require a "lead compound" analysis, Columbia's claim is nevertheless obvious under the "lead compound" approach.

The first step in a "lead compound" analysis is identifying a lead compound. A lead compound need not be the single best compound in the prior art. *Aluma*

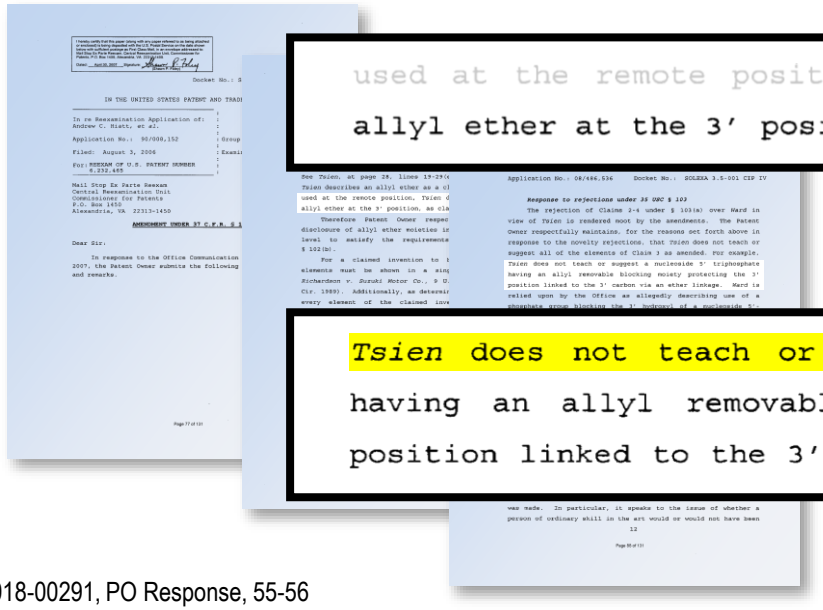
-42-

Illumina's Current Position

D. Tsien's 3′-O-allyl dATP is a Lead Compound

IPR2018-00291, Petition, 42

Illumina's Previous Position



used at the remote position, *Tsien* does not describe using an allyl ether at the 3′ position, as claimed.

Ex. 2065, 86

Tsien does not teach or suggest a nucleoside 5′ triphosphate having an allyl removable blocking moiety protecting the 3' position linked to the 3′ carbon via an ether linkage. *Ward* is

Ex. 2065, 88

IPR2018-00291, PO Response, 55-56

Illumina's Motivation to Select -CH_2-CH=CH_2 Capping Group Is Based Solely on Tsien

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UNITED STATES PATENT AND TRADEMARK OFFICE

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THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK
Patent Owner.

Case No. IPR2018-00291
U.S. Patent No. 9,718,852

PETITION FOR
U.S. PA

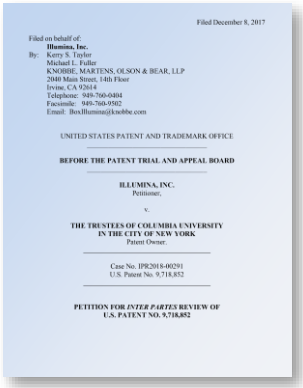
The Board and Federal Circuit determined a POSA reading Tsien would clearly envisage a nucleotide having a fluorescent label attached via a cleavable linker and a 3′-OH capping group. Ex-1005 at 10; Ex-1008 at 31. Tsien discloses a nucleotide analogue having a fluorescent label attached via a cleavable linker to a base and a 3′-OH capping group. Tsien discloses that a 3′-O-allyl is an advantageous capping group because "deblocking occurs only when the specific deblocking reagent is present and premature deblocking during incorporation is minimized." Ex-1013 at 24:29-25:3. Tsien's disclosure of the desirability of the allyl protecting group would have motivated a POSA to select and use the 3′-O-allyl capping group on the nucleotide analogues disclosed by Tsien. Indeed, combining favorable disclosures within Tsien "does not require a leap of inventiveness." *Boston Sci. Scimed, Inc. v. Cordis Corp.*, 554 F.3d 982, 991 (Fed. Cir. 2009); Ex-1012 ¶¶94-95. Thus, based on Tsien's disclosure, a POSA would have been motivated to use Tsien's 3′-O-allyl group in Tsien's sequencing methods.

Cir. 2009); Ex-1012 ¶¶94-95. Thus, based on Tsien's disclosure, a POSA would have been motivated to use Tsien's 3′-O-allyl group in Tsien's sequencing methods.

IPR2018-00291, Petition, 34

IPR2018-00291, Sur-Reply, 8; PO Response, 54

The "Non-Base Hydrolysis" Advantage Alleged in the Petition Does Not Exist for Allyl Ethers



Filed December 8, 2017

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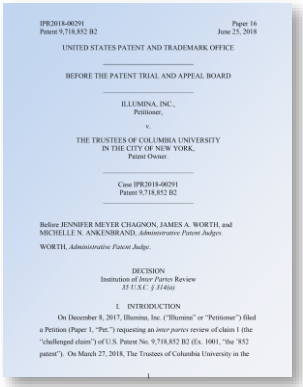
Case No. IPR2018-00291
U.S. Patent No. 9,718,852

PETITION FOR *INTER PARTES* REVIEW OF U.S. PATENT NO. 9,718,852



IPR2018-00291
Patent 9,718,852 B2

Paper 16
June 25, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

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THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,
Patent Owner.

Case IPR2018-00291
Patent 9,718,852 B2

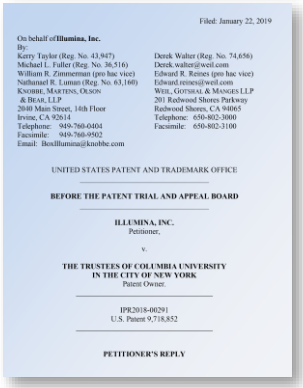
Before JENNIFER MEYER CHAGNON, JAMES A. WORTH, and MICHELLE N. ANKENBRAND, *Administrative Patent Judges*.

WORTH, *Administrative Patent Judge*.

DECISION
Institution of *Inter Partes* Review
35 U.S.C. § 314(a)

I. INTRODUCTION

On December 8, 2017, Illumina, Inc. ("Illumina" or "Petitioner") filed a Petition (Paper 1, "Pet.") requesting an *inter partes* review of claim 1 (the "challenged claim") of U.S. Patent No. 9,718,852 B2 (Ex. 1001, "the '852 patent"). On March 27, 2018, The Trustees of Columbia University in the



Filed: January 22, 2019

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UNITED STATES PATENT AND TRADEMARK OFFICE

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IPR2018-00291
U.S. Patent 9,718,852

PETITIONER'S REPLY

Petition

Tsien discloses that the allyl group is advantageous because it is "cleaved selectively using chemical procedures other than base hydrolysis ... [which has] some advantages over groups cleavable by base hydrolysis—deblocking occurs

IPR2018-00291, Petition, 21-22 (citing Gigg and Warren)

Institution Decision

(using "well-established synthetic procedures"). Based on the evidence at this stage of the proceeding, we are persuaded that Petitioner has made a sufficient showing that a person of ordinary skill would have combined the teachings of Tsien to use the allyl group that Tsien discloses to be an advantageous blocking group, to block the 3'-oxygen, as elsewhere disclosed in Tsien's method. *See* Ex. 1013, 12:27–29, 24:5–25:3. We invite further

IPR2018-00291, Institution Dec., 13-14

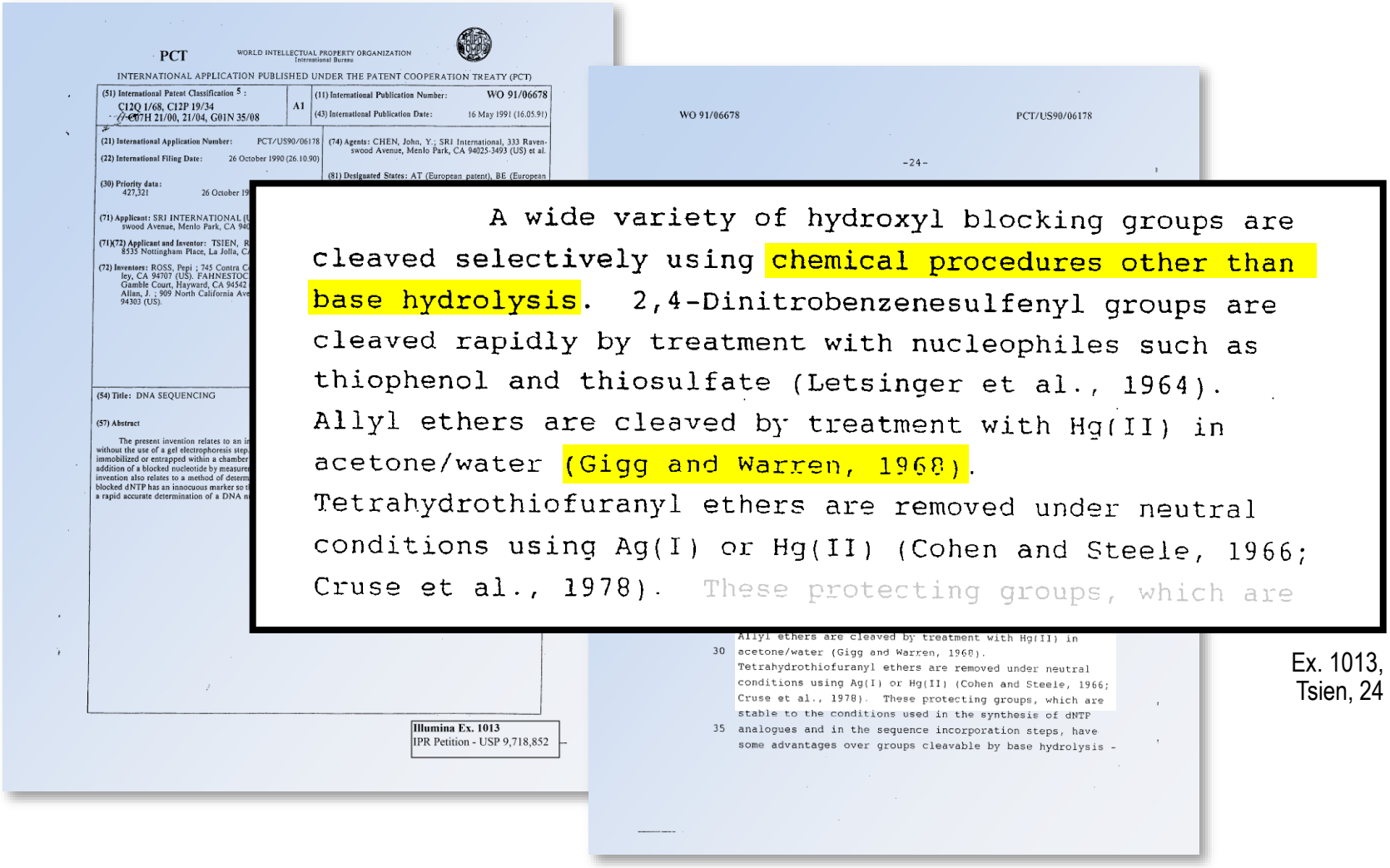
Reply

cleavage. Tsien cites to the pioneering 1968 Gigg article, which discloses that allyl ether is cleavable by base hydrolysis. Ex. 1013, 24:29-30; Ex. 1046, 1905-06. Tsien

IPR2018-00291, Reply, 19

IPR2018-00291, Sur-Reply, 8; PO Response, 54

Illumina's Belated Argument That Tsien Additionally Teaches Non-Base Hydrolysis Is Unsupported



PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 5: C12Q 1/68, C12P 19/34, C07H 21/00, 21/04, G01N 35/08 — A1

(11) International Publication Number: WO 91/06678

(43) International Publication Date: 16 May 1991 (16.05.91)

(21) International Application Number: PCT/US90/06178

(22) International Filing Date: 26 October 1990 (26.10.90)

(74) Agents: CHEN, John, Y.; SRI International, 333 Ravenswood Avenue, Menlo Park, CA 94025-3493 (US) et al.

(81) Designated States: AT (European patent), BE (European

(30) Priority data: 427,321 26 October 19

(71) Applicant: SRI INTERNATIONAL [U Swood Avenue, Menlo Park, CA 940

(71)(72) Applicant and Inventor: TSIEN, R 8535 Nottingham Place, La Jolla, CA

(72) Inventors: ROSS, Pepi ; 745 Contra C ley, CA 94707 (US). FAHNESTOC Gamble Court, Hayward, CA 94542 Allan, J. ; 909 North California Ave 94303 (US).

(54) Title: DNA SEQUENCING

(57) Abstract

The present invention relates to an in without the use of a gel electrophoresis step immobilized or entrapped within a chamber addition of a blocked nucleotide by measure invention also relates to a method of determ blocked dNTP has an innocuous marker so t a rapid accurate determination of a DNA n

Illumina Ex. 1013
IPR Petition - USP 9,718,852

WO 91/06678 PCT/US90/06178

-24-

A wide variety of hydroxyl blocking groups are cleaved selectively using chemical procedures other than base hydrolysis. 2,4-Dinitrobenzenesulfenyl groups are cleaved rapidly by treatment with nucleophiles such as thiophenol and thiosulfate (Letsinger et al., 1964). Allyl ethers are cleaved by treatment with Hg(II) in acetone/water (Gigg and Warren, 1968). Tetrahydrothiofuranyl ethers are removed under neutral conditions using Ag(I) or Hg(II) (Cohen and Steele, 1966; Cruse et al., 1978). These protecting groups, which are

Allyl ethers are cleaved by treatment with Hg(II) in
30 acetone/water (Gigg and Warren, 1968).
Tetrahydrothiofuranyl ethers are removed under neutral
conditions using Ag(I) or Hg(II) (Cohen and Steele, 1966;
Cruse et al., 1978). These protecting groups, which are
stable to the conditions used in the synthesis of dNTP
35 analogues and in the sequence incorporation steps, have
some advantages over groups cleavable by base hydrolysis -

Ex. 1013, Tsien, 24

IPR2018-00291, Sur-Reply, 8; PO Response, 54

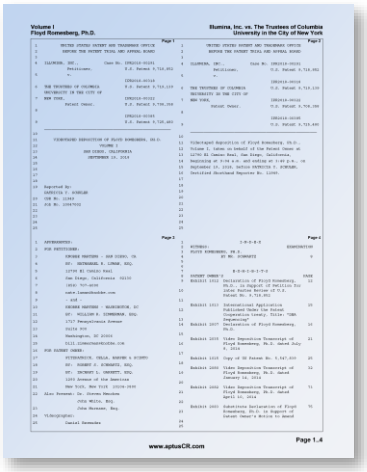
Illumina's Dower-Based Arguments Fail

Relevant to:

IPR2018-00291, 318, 322, 385: Ground 2

IPR2018-00797: Ground 3

Dower's Method Is SBS

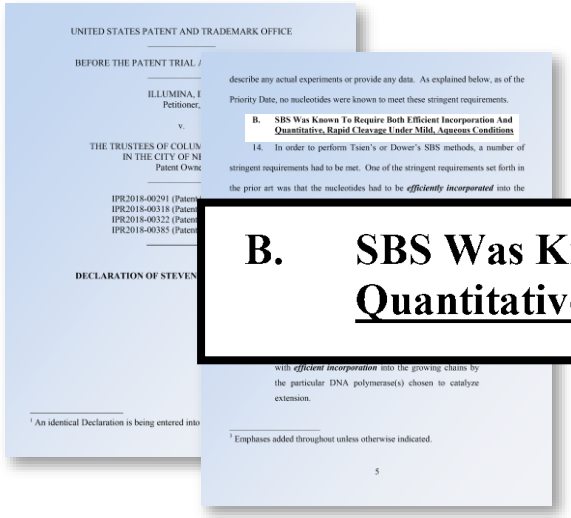


Floyd Romesberg, Ph.D.
Deposition

Q. And the Dower patent relates to SBS, correct?

A. That is correct.

Ex. 2126, Romesberg Dep., 25:15-17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describe any actual experiments or provide any data. As explained below, as of the Priority Date, no nucleotides were known to meet these stringent requirements.

B. SBS Was Known To Require Both Efficient Incorporation And Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

14. In order to perform Tsien's or Dower's SBS methods, a number of stringent requirements had to be met. One of the stringent requirements set forth in the prior art was that the nucleotides had to be ***efficiently incorporated*** into the

with ***efficient incorporation*** into the growing chains by the particular DNA polymerase(s) chosen to catalyze extension.

[1] An identical Declaration is being entered into

[2] Emphases added throughout unless otherwise indicated.

5

B. SBS Was Known To Require Both Efficient Incorporation And Quantitative, Rapid Cleavage Under Mild, Aqueous Conditions

Ex. 2116, Menchen Decl., 5

IPR2018-00291, PO Response, 5-6; Opp. Mot. Exclude, 9-10

Dower's SBS Method Requires Efficient Incorporation

US005547839A

United States Patent [19]

Dower et al.

[11] **Patent Number: 5,547,839**

[45] **Date of Patent: Aug. 20, 1996**

[54] **SEQUENCING OF SURFACE IMMOBILIZED POLYMERS UTILIZING MICROFLOURESCENCE DETECTION**

[75] Inventors: **William J. Dower**, Menlo Park; **Stephen P. A. Fodor**, Palo Alto, both of Calif.

[73] Assignee: **Affymax Technologies N.V.**, Curacao, Netherlands Antilles

[21] Appl. No.: **626,730**

[22] Filed: **Dec. 6, 1990**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 492,462, Mar. 7, 1990, Pat. No. 5,143,854, which is a continuation-in-part of Ser. No. 362,901, Jun. 7, 1989, abandoned.

[51] **Int. Cl.**[6] **C12Q 1/68**

[52] **U.S. Cl.** **435/6**; 536/24.33; 536/24.3

[58] **Field of Search** 435/6, 91, 810, 435/973, 975, 91.2; 436/527, 530, 531, 56, 94, 800, 808; 536/27, 24.33; 935/77

[56] **References Cited**

U.S. PATENT DOCUMENTS

4,542,102	9/1985	Dattagupta et al.	435/6
4,582,789	4/1986	Sheldon et al.	435/6
4,656,127	4/1987	Mundy	435/6
4,689,405	8/1987	Frank et al.	536/27
4,713,326	12/1987	Dattagupta et al.	435/6
4,855,225	8/1989	Fung et al.	435/6
4,889,818	12/1989	Gelfand et al.	435/194
4,962,037	10/1990	Jett et al.	435/6
4,965,188	10/1990	Mullis et al.	435/6
5,002,867	3/1991	Macevicz	435/6
5,026,840	6/1991	Dattagupta et al.	536/27
5,075,216	12/1991	Innis et al.	435/6
5,126,239	6/1992	Livak et al.	435/6
5,143,854	9/1992	Pirrung et al.	436/518

FOREIGN PATENT DOCUMENTS

392546	10/1990	European Pat. Off. .	
2233654	1/1991	United Kingdom .	
9013666	5/1990	WIPO	C12Q 1/68
9015070	12/1990	WIPO .	
9107087	5/1991	WIPO .	
9106678	5/1991	WIPO	C12Q 1/68

OTHER PUBLICATIONS

Ikehara et al., "The Synthesis of Polynucleotides," *Advances in Carbohydrate Chemistry and Biochemistry* (1979) 36:135–213.

Tsugita et al., "Sensitization of Edman Amino Acid Derivatives Using the Flourescent Reagent, 4–Aminofluorescein," *J. Biochem.* (1989) 106:60–65.

Ross et al., "Interstrand Crosslinks due to 4,5',8–Trimethyl–psoralen and Near Ultraviolet Light in Specific Sequences of Animal DNA," *J. Mol. Biol.* (1988) 201:339–351.

Barinaga, 27 Sep. 1991, Science 253:1489.

Fodor et al., 15 Feb. 1991, Science 251:767–773.

Dower and Fodor, 1991, Ann. Rep. Med. Chem. 26:271–280.

Little, 16 Aug. 1990, Nature 346:611–612.

Craig et al., 1990, Nucl. Acids. Res. 18:2653–2660.

Chatterjee et al., 1990, J. Am. Chem. Soc. 112:6397–6399.

Pfeifer et al., 10 Nov. 1989, Science 246:810–812.

Evans et al., Jul. 1989, Proc. Natl. Acad. Sci. USA 86:5030–4.

Saiki and Gelfand, 1989, Amplifications 1:4–6.

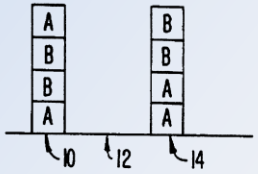
(List continued on next page.)

Primary Examiner—W. Gary Jones
Assistant Examiner—Scott Houtteman
Attorney, Agent, or Firm—Townsend & Townsend & Crew LLP

[57] **ABSTRACT**

Means for simultaneous parallel sequence analysis of a large number of biological polymer macromolecules. Apparatus and methods may use fluorescent labels in repetitive chemistry to determine terminal monomers on solid phase immobilized polymers. Reagents which specifically recognize terminal monomers are used to label polymers at defined positions on a solid substrate.

7 Claims, 10 Drawing Sheets



Illumina Ex. 1015
IPR Petition - USP 9,718,852

25

nents appropriate to the particular DNA polymerase selected, are placed in the incubation chamber in direct contact with the surface.

Step 2: Fluorescent chain terminators (analogs of dATP, dCTP, dGTP, and TP, each labeled with fluorophore preferably emitting at a distinguishable wavelength) are added to the reaction at a sufficient concentration and under suitable reaction conditions (time, temperature, pH, ionic species, etc., See Sambrook et al. (1989) *Molecular Cloning*, vols. 1–3, and Prober et al.) to cause essentially all of the chains on the surface to be extended by one base and thereby terminated. Detection of the specific label thereby incorporated into each chain identifies the last base added at each positional address in the matrix.

Step 3: The chain termination should be reversible by some means, such as treatment with light, heat, pH, certain other chemical or biological (enzymatic) reagents, or some combination of these. Typically the chain termination results from a blocking moiety which is labile to mild treatment. By one of these means, the blocked 3'OH of the terminating base must be made available for chain extension in the next round of polymerization.

Step 4: There are several suitable labeled, terminator structures as follows:

(a) The fluorophore itself functions as the chain terminator by placement on the 3' hydroxyl through a linkage that

26

followed by scanning detection. After all four fluorophores are added, reversal of the termination is performed, allowing for the addition of the next base analog. Then, each scanning step determines whether the immediately preceding labeled nucleotide had been incorporated at each distinct position.

The structures of the fluorescently labeled and reversible terminator base analogs are selected to be compatible with efficient incorporation into the growing chains by the particular DNA polymerase(s) chosen to catalyze extension. For example, where two different chain terminators are used, they may be utilized by two different polymerases that are both present during the chain extension step.

Step 5: An optional step is the permanent capping of chain extension failures with high concentrations of dideoxynucleotide triphosphates. This step serves to reduce the background of fluorescence caused by addition of an incorrect base because of inefficient chain extension (termination) at an earlier step.

Step 6: After scanning to determine fluorescence, the fluorophore is removed or deactivated. Deactivation of the fluorophore can be achieved by a photodestruction event. The chain elongation block is reversed (usually by removing a blocking group to expose the 3'OH) by suitable methods that depend on the particular base analogs chosen; and the substrate is washed in preparation for the next round of polymerization.

nucleotide triphosphate analogs as described by Prober et al. (1987) *Science* 238:336–341. A second, unlabeled and reversible, set of terminators is also required. Examples of these compounds are deoxynucleotide triphosphates with small blocking groups such as acetyl, tBOC, NBOC and NVOC on the 3'OH. These groups are easily and efficiently removed under conditions of high or low pH, exposure to light or heat, etc. After each round of base addition and detection, the fluorophores are deactivated by exposure to light under suitable conditions (these chains have their labeling moiety destroyed and remain terminated, taking part in no further reactions). The unlabeled, reversible terminators are unblocked at the 3'OH by the appropriate treatment to allow chain extension in subsequent rounds of elongation. The proportion of chains labeled in each round can be controlled by the concentration ratio of fluorescent to non-fluorescent terminators, and the reaction can be driven to completion with high concentrations of the unlabeled terminators.

(d) A single dye strategy is used where all the base analog terminators carry the same fluorophore and each is added one at a time: A, C, G, T. The addition of each base is

The DNA will usually be attached to the substrate by the 3' or 5' terminus depending on the scheme of labeling and cleavage. Because there are well-known 5'-labeling methods, see, e.g., Gait (1984) *Oligonucleotide Synthesis: A Practical Approach*, IRL Press, Oxford, this discussion will assume the 3' end is attached to the substrate with the 5' end free.

Step 1: All the 5'-end bases are labeled with 5'-specific chemistry, e.g., 5' amino linkage to FITC, Nelson et al. (1989) *Nucl. Acids Res.* 17:7179–7186, which is hereby incorporated herein by reference.

Step 2: Scan the matrix to obtain the background level.

Step 3: Optional: Cap all of the labeling failures, e.g., polymers whose ends were not labeled.

Step 4: The terminal A's are removed with end-base, A-specific reagents (such a reagent may be chemical or biological). One example is a 5'-fluorescein-dAMP-specific exonuclease made as a catalytic antibody (see the description above for a scheme of producing this reagent).

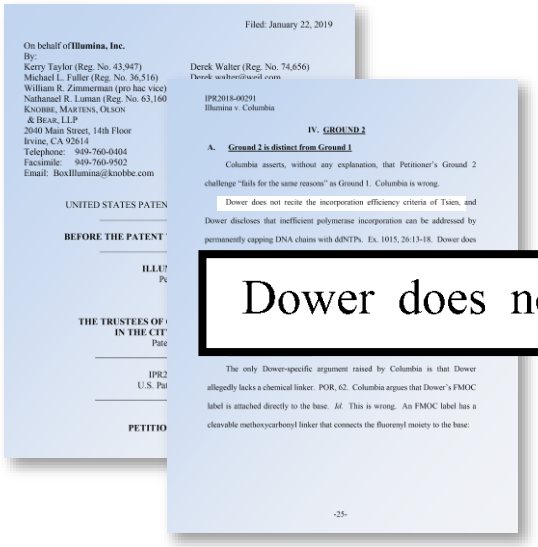
Step 5: Scan the matrix to detect those chains that had terminated in A (these will be reduced in fluorescence compared to the fluorescently labeled background).

The structures of the fluorescently labeled and reversible terminator base analogs are selected to be compatible with efficient incorporation into the growing chains by the particular DNA polymerase(s) chosen to catalyze extension.

Ex. 1015, Dower, 26:6-9

IPR2018-00291, PO Response, 3-6; Sur-Reply, 26-27

Illumina's Belated Position That Dower Does Not Require Efficient Incorporation Is Belied by Its Petition


Filed: January 22, 2019

On behalf of Illumina, Inc.

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Michael L. Fuller (Reg. No. 36,516)
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Derek Walter (Reg. No. 74,656)

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BEFORE THE PATENT

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IPR2018-00291
Illumina v. Columbia

IV. GROUND 2

A. Ground 2 is distinct from Ground 1

Columbia asserts, without any explanation, that Petitioner's Ground 2 challenge "fails for the same reasons" as Ground 1. Columbia is wrong.

Dower does not recite the incorporation efficiency criteria of Tsien, and Dower discloses that inefficient polymerase incorporation can be addressed by permanently capping DNA chains with ddNTPs. Ex. 1015, 26:13-18. Dower does

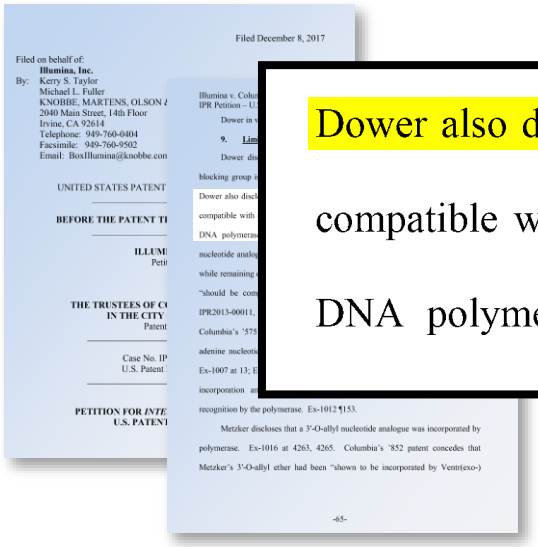
The only Dower-specific argument raised by Columbia is that Dower allegedly lacks a chemical linker. POR, 62. Columbia argues that Dower's FMOC label is attached directly to the base. *Id.* This is wrong. An FMOC label has a cleavable methoxycarbonyl linker that connects the fluorenyl moiety to the base:

-25-

Illumina's Current Position

> Dower does not recite the incorporation efficiency criteria of Tsien,

IPR2018-00291, Reply, 25


Filed December 8, 2017

Filed on behalf of:
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UNITED STATES PATENT

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IN THE CITY
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Case No. IP
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IPR2013-00011,

Columbia's '575

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Ex-1007 at 13; E

incorporation a

recognition by the polymerase. Ex-1012 ¶153.

Metzker discloses that a 3'-O-allyl nucleotide analogue was incorporated by polymerase. Ex-1016 at 4263, 4265. Columbia's '852 patent concedes that Metzker's 3'-O-allyl ether had been "shown to be incorporated by Ventr(exo-)

-65-

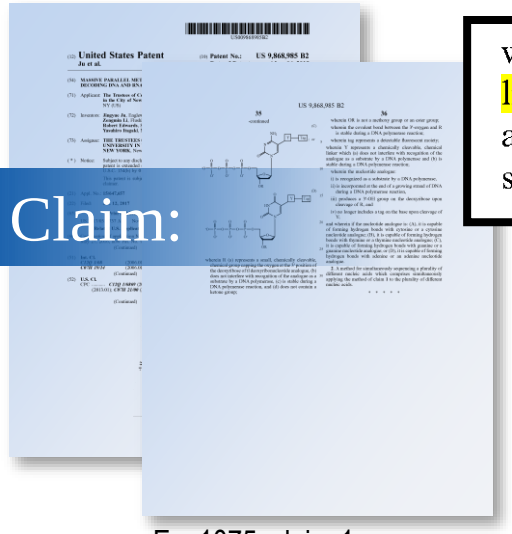
Illumina's Previous Position

> Dower also discloses that the label and 3′-OH blocking group "are selected to be compatible with efficient incorporation into the growing chains by the particular DNA polymerase(s) chosen to catalyze extension." *Id.* at 26:6-9. Dower's

IPR2018-00291, Petition, 65

IPR2018-00291, Sur-Reply, 26-27

Dower/Prober Does Not Describe the Claimed Cleavable Linker "Y"

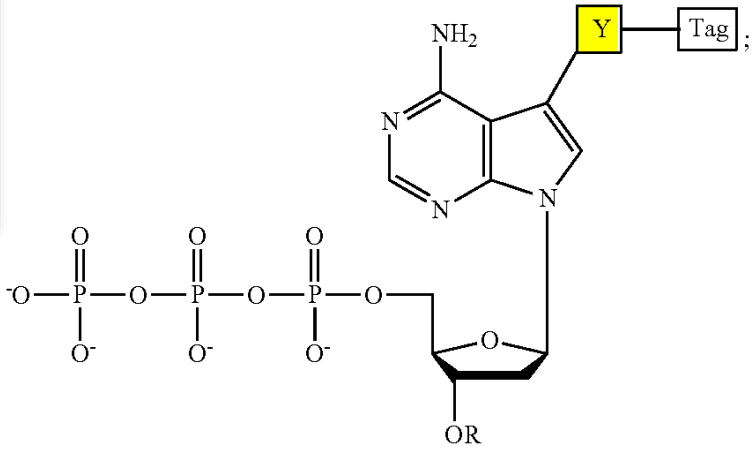


Ex. 1075, claim 1

Claim:

wherein Y represents a chemically cleavable, chemical linker which (a) does not interfere with recognition of the analogue as a substrate by a DNA polymerase and (b) is stable during a DNA polymerase reaction;

(B)



Dower: **No Y**

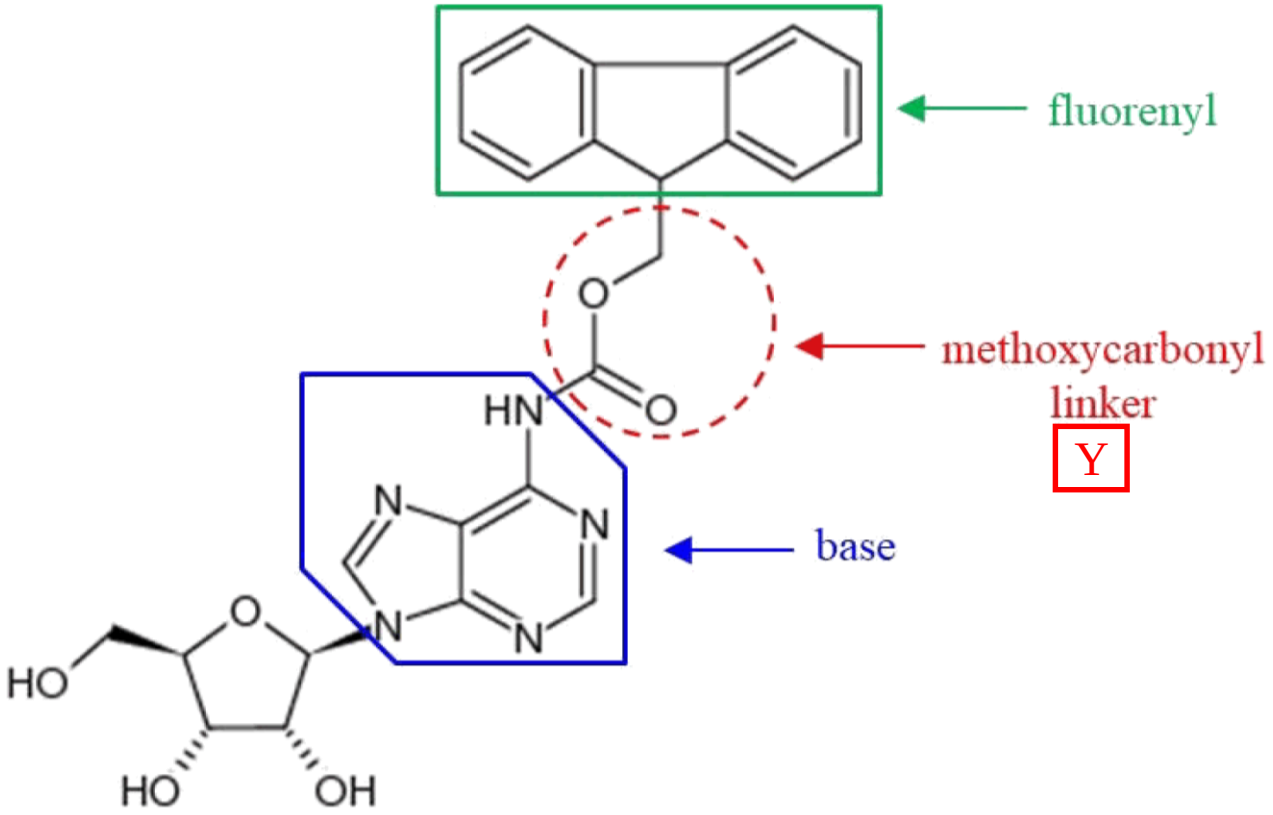
Ex. 2116, Menchen Decl., ¶111

Prober: **No cleavable Y**

Ex. 2113, Romesberg Dep., 124:11-15
Ex. 2116, Menchen Decl., ¶111

IPR2018-00291, PO Response, 61-62; Sur-Reply, 22-24

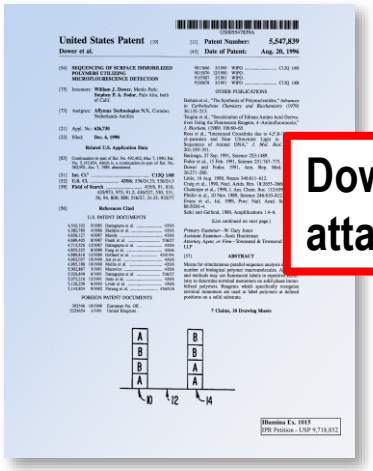
Illumina Belatedly Alleges Dower's FMOC Has Built-In Cleavable Linker



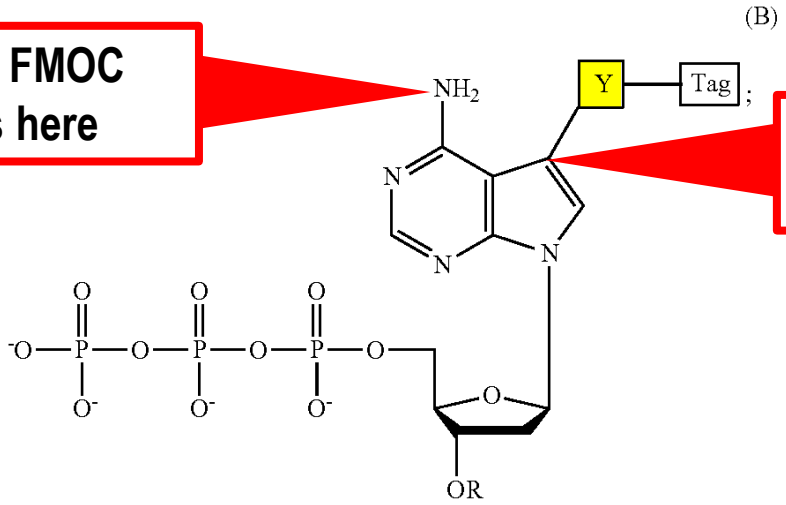
IPR2018-00291, Reply, 26

IPR2018-00291, Sur-Reply, 22-24

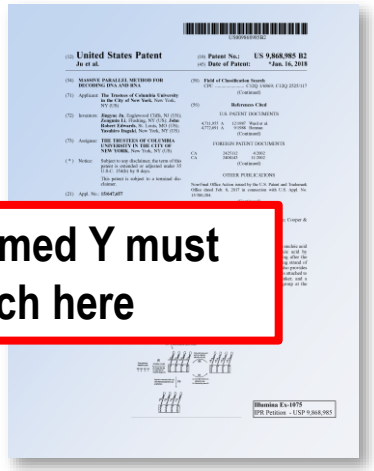
Dower's FMOC Attaches to Different Site Than Claimed Y



Dower's FMOC attaches here



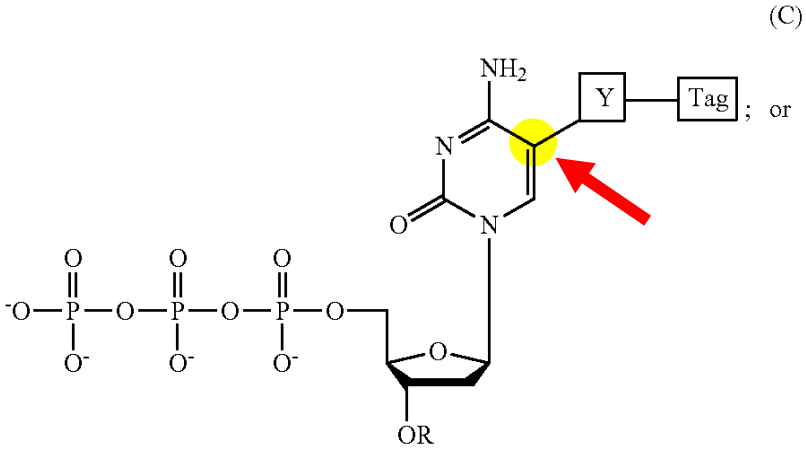
Claimed Y must attach here



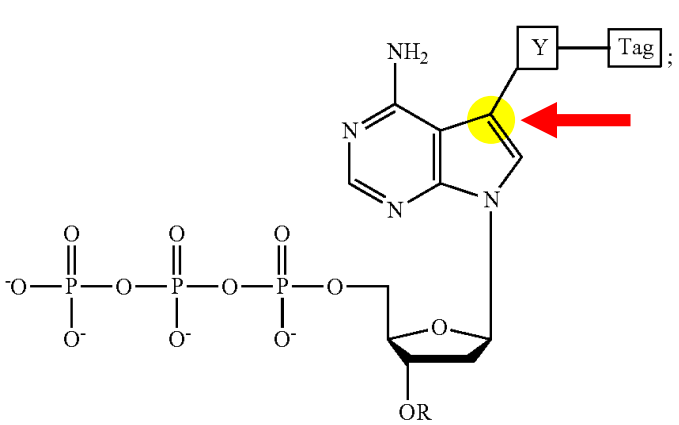
Ex. 1075, claim 1

Dower's FMOC Attaches to Different Site Than Claimed Y

Claimed linker "Y" on 5-pyrimidine carbon

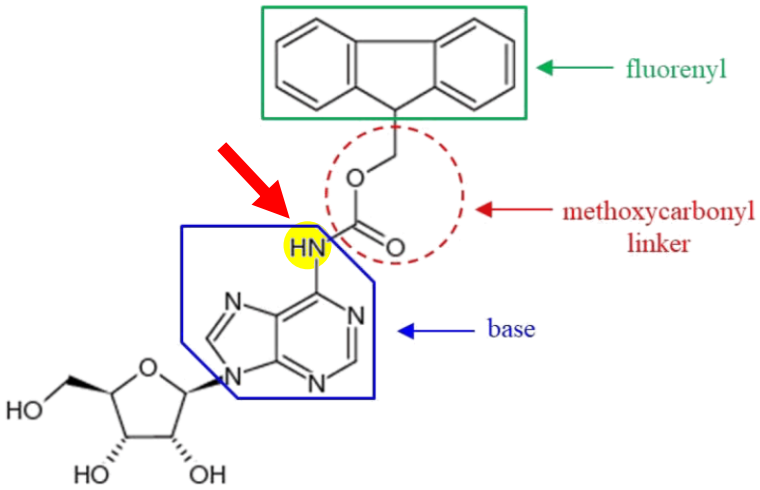


Claimed linker "Y" on 7-deazapurine carbon



Ex. 1075, '985 Patent, claim 1

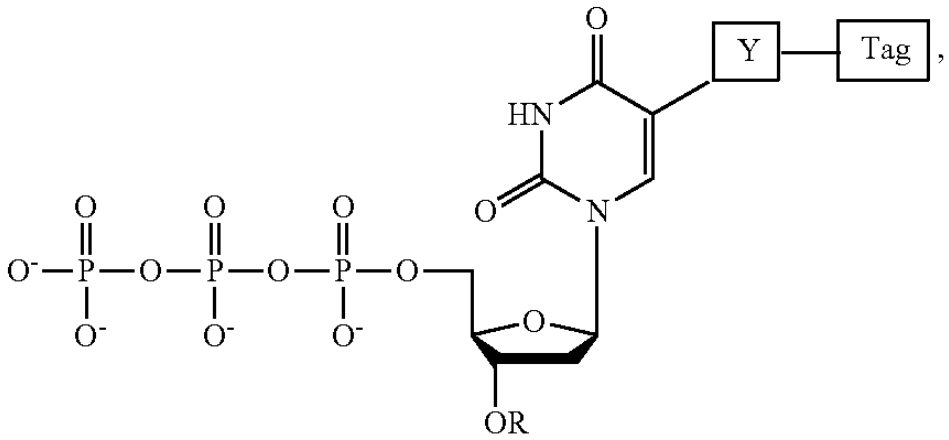
Dower FMOC on exocyclic amine



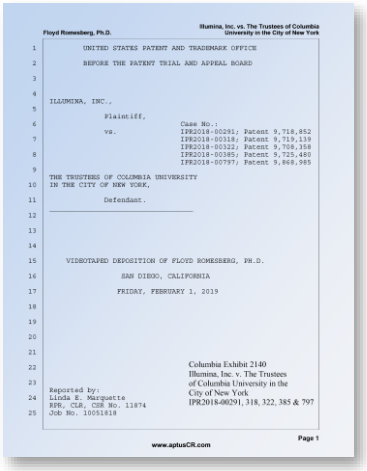
IPR2018-00291, Reply, 26

IPR2018-00291, Sur-Reply, 22-24

Dower's FMOC Cannot Attach to Thymine Because It Lacks an Exocyclic Amine



Ex. 1003, '139 Patent, claim 1


Illumina, Inc. vs. The Trustees of Columbia University in the City of New York
Floyd Romesberg, Ph.D.

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,

Plaintiff,

vs.

Case No.:
IPR2018-00291; Patent 9,718,852
IPR2018-00318; Patent 9,719,139
IPR2018-00322; Patent 9,708,358
IPR2018-00385; Patent 9,725,480
IPR2018-00797; Patent 9,868,985

THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,

Defendant.

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.

SAN DIEGO, CALIFORNIA

FRIDAY, FEBRUARY 1, 2019

Columbia Exhibit 2140
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291, 318, 322, 385 & 797

Reported by:
Linda E. Marquette
RPR, CLR, CSR No. 11874
Job No. 10051818

www.aptusCR.com

Page 1

Floyd Romesberg, Ph.D.
Deposition

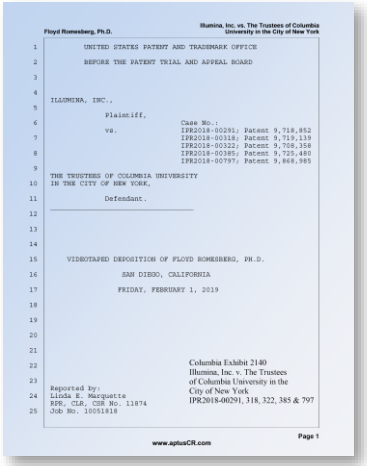
Q. But Dower doesn't teach attaching FMOC to the three bases that he teaches attaching it to through any kind of additional linker, correct?

A. Dower gives an example where he uses FMOC to attach directly to the three of the four that you could directly attach to. T is not.

Ex. 2140, Romesberg Dep., 197:16-21

IPR2018-00291, Sur-Reply, 22-24

Illumina Presents No Evidence That Chemistry Existed to Attach FMOC to Claimed Position


Floyd Romesberg, Ph.D. — Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

UNITED STATES PATENT AND TRADEMARK OFFICE

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ILLUMINA, INC.,

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Case No.:
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IPR2018-00322; Patent 9,708,358
IPR2018-00385; Patent 9,725,480
IPR2018-00797; Patent 9,868,985

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Linda E. Marquette
RPR, CLR, CSR No. 11874
Job No. 10051810

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Page 1

Floyd Romesberg, Ph.D.
Deposition

Q. You haven't cited any references in any declarations in this IPR -- these IPRs with conditions where you could attach FMOC to the 5 position, correct?

A. No. You asked me if I thought you could. And I'm simply speculating.

Q. Okay. But you haven't cited any.

A. No, nor do I know of any.

Q. Okay. And the same for the diaza position, attaching it to the diaza position of a 7 –

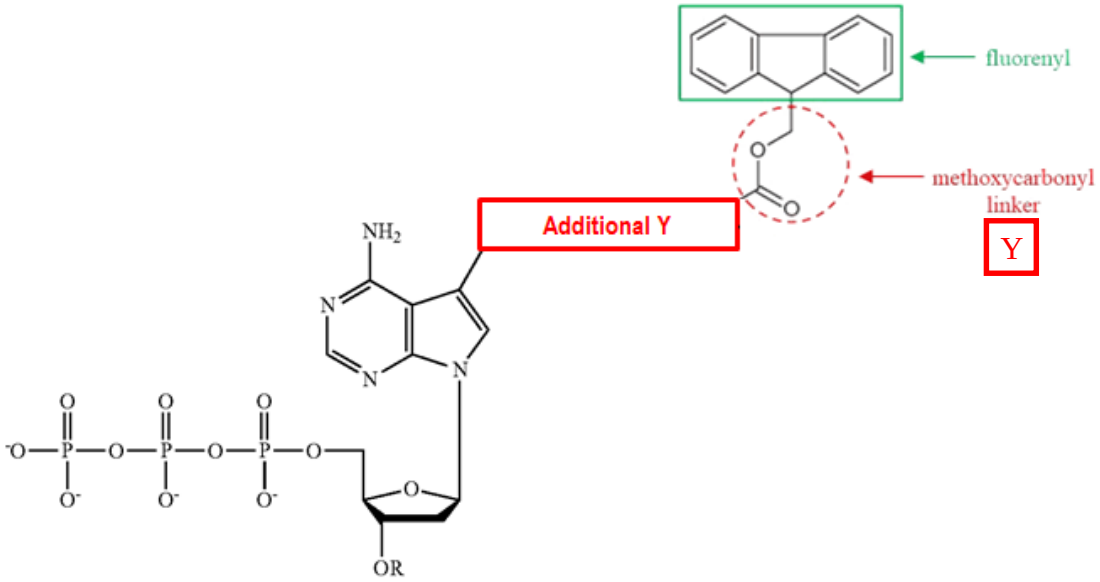
…

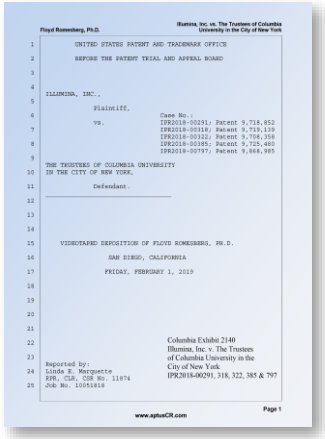
A. I did not.

Ex. 2140, Romesberg Dep., 201:8-23

IPR2018-00291, Sur-Reply, 22-24

Illumina's Most Recent Position Requires Adding Another Linker





Floyd Romesberg, Ph.D.
Deposition

Q. But Dower doesn't teach attaching Fmoc to the three bases that he teaches attaching it to through any kind of additional linker, correct?

A. …He didn't attach the Fmoc directly, but it would be an exocyclic amine that you would exactly be able to do that if you chose. Or you would be able to choose an additional linker between them. And that's what Seitz did.

Ex. 2140, Romesberg Dep., 197:16-198:8

IPR2018-00291, Sur-Reply, 22-24

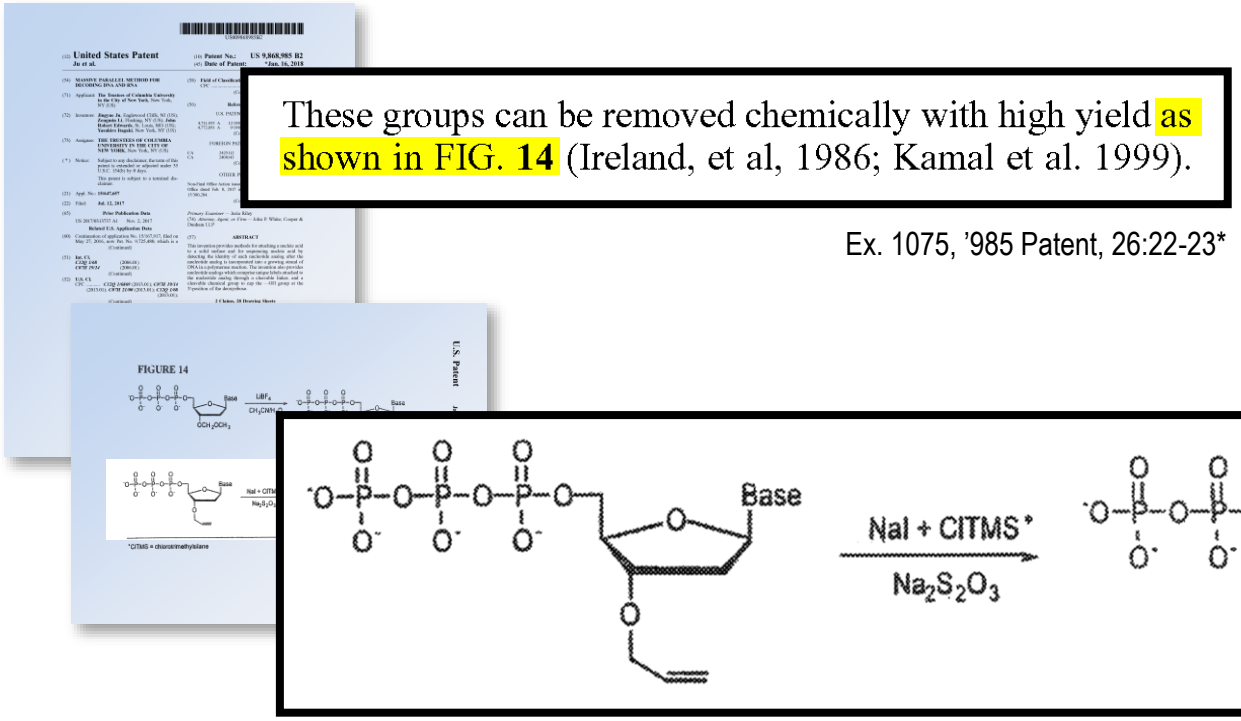
Columbia Did Not Admit That Kamal Teaches SBS-Compatible Cleavage Conditions

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

Columbia's Statements Concerning Allyl Protecting Groups Are Not Admissions of Prior Art Teachings



Ex. 1075, '985 Patent, 26:22-23*

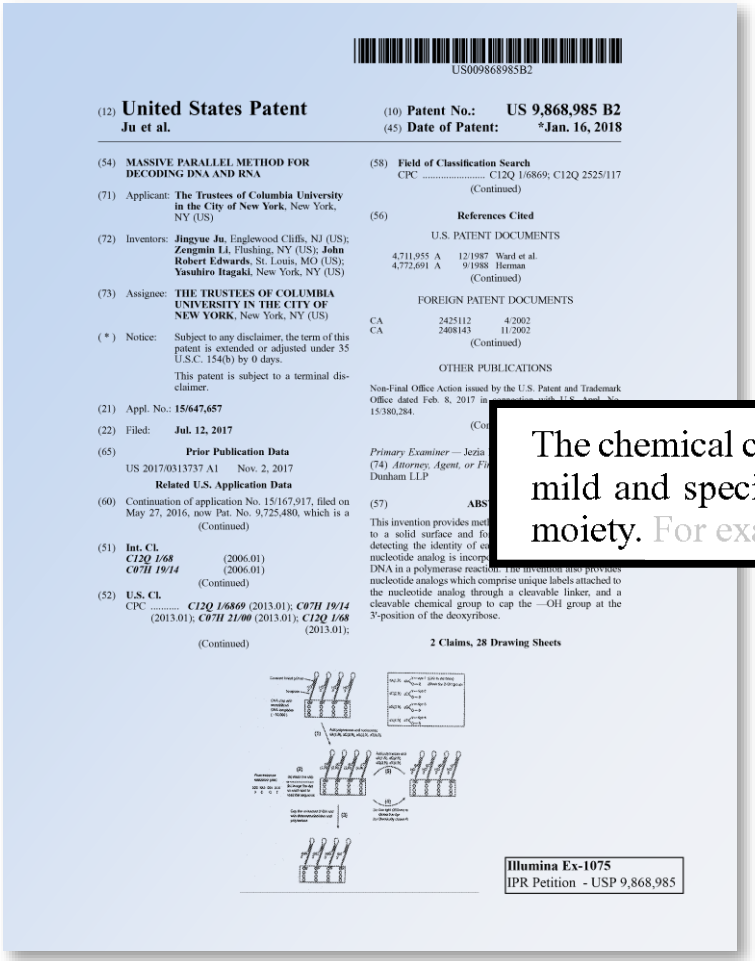
Ex. 1075, '985 Patent, Fig. 14

FIGURE 14 is Not in Kamal

IPR2018-00291, Sur-Reply, 17; PO Response, 36; Ex. 1112, 126:9-127:8, 130:18-24, 233:2-235:10

* References incorporated in their entirety, Ex. 1075, 1:35-38

Columbia Does Not Cite Kamal for Mild and Specific Conditions



US009868985B2

(12) **United States Patent**
Ju et al.

(10) **Patent No.: US 9,868,985 B2**
(45) **Date of Patent: *Jan. 16, 2018**

(54) **MASSIVE PARALLEL METHOD FOR DECODING DNA AND RNA**

(71) Applicant: **The Trustees of Columbia University in the City of New York**, New York, NY (US)

(72) Inventors: **Jingyue Ju**, Englewood Cliffs, NJ (US); **Zengmin Li**, Flushing, NY (US); **John Robert Edwards**, St. Louis, MO (US); **Yasuhiro Itagaki**, New York, NY (US)

(73) Assignee: **THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK**, New York, NY (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

This patent is subject to a terminal disclaimer.

(21) Appl. No.: **15/647,657**

(22) Filed: **Jul. 12, 2017**

(65) **Prior Publication Data**

US 2017/0313737 A1 Nov. 2, 2017

Related U.S. Application Data

(60) Continuation of application No. 15/167,917, filed on May 27, 2016, now Pat. No. 9,725,480, which is a
(Continued)

(51) **Int. Cl.**
C12Q 1/68 (2006.01)
C07H 19/14 (2006.01)
(Continued)

(52) **U.S. Cl.**
CPC ***C12Q 1/6869*** (2013.01); ***C07H 19/14*** (2013.01); ***C07H 21/00*** (2013.01); ***C12Q 1/68*** (2013.01);
(Continued)

(58) **Field of Classification Search**
CPC C12Q 1/6869; C12Q 2525/117
(Continued)

(56) **References Cited**

U.S. PATENT DOCUMENTS

4,711,955 A 12/1987 Ward et al.
4,772,691 A 9/1988 Herman
(Continued)

FOREIGN PATENT DOCUMENTS

CA 2425112 4/2002
CA 2408143 11/2002
(Continued)

OTHER PUBLICATIONS

Non-Final Office Action issued by the U.S. Patent and Trademark Office dated Feb. 8, 2017 in connection with U.S. Appl. No. 15/380,284.
(Con

Primary Examiner — Jezia
(74) *Attorney, Agent, or Fir*
Dunham LLP

(57) **ABS**

This invention provides meth
to a solid surface and fo
detecting the identity of ea
nucleotide analog is incorp
DNA in a polymerase reaction. The invention also provides nucleotide analogs which comprise unique labels attached to the nucleotide analog through a cleavable linker, and a cleavable chemical group to cap the —OH group at the 3'-position of the deoxyribose.

2 Claims, 28 Drawing Sheets

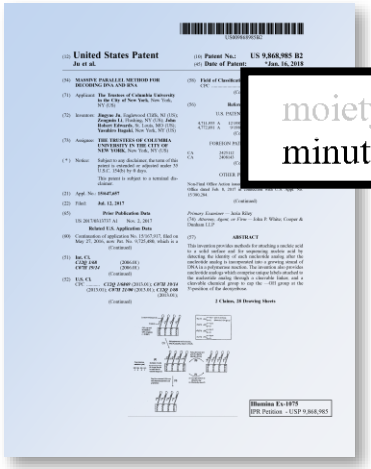
Illumina Ex-1075
IPR Petition - USP 9,868,985

The chemical cleavage of the MOM and allyl groups is fairly mild and specific, so as not to degrade the DNA template moiety. For example, the cleavage of the allyl group takes 3

Ex. 1075, '985 Patent, 26:24-26

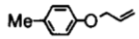
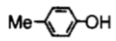
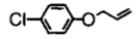
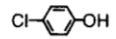
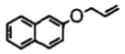
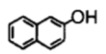
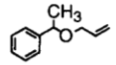
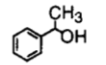
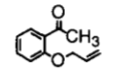
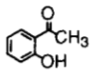
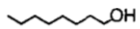
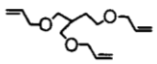
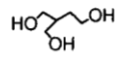
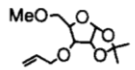
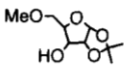
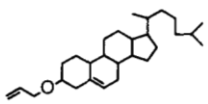
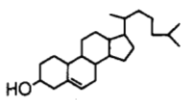
IPR2018-00291, Sur-Reply, 17; Ex. 1112, 121:3-122:4

Columbia Admitted Only That Kamal Taught 93% Cleavage of Non-Nucleotide Protected Alcohols Under Kamal's Conditions



moiety. For example, the cleavage of the allyl group takes 3 minutes with more than 93% yield (Kamal et al. 1999),

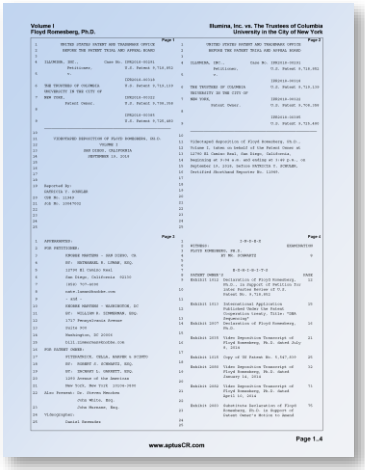
Ex. 1075, '985 Patent, 26:26-27

Entry	Substrate	Time(min)	Product	Yield(%)
1		2		98
2		2		98
3		2		98
4		3		96
5		5		90
6		3		95
7		3		95[16]
8		2		93
9		3		93

Ex. 1037, Kamal, 372

IPR2018-00291, Sur-Reply, 17; PO Response, 36; Ex. 1112, 126:9-127:8, 130:18-24, 233:2-235:10

Kamal's 93% Cleavage Is Not Useful For SBS



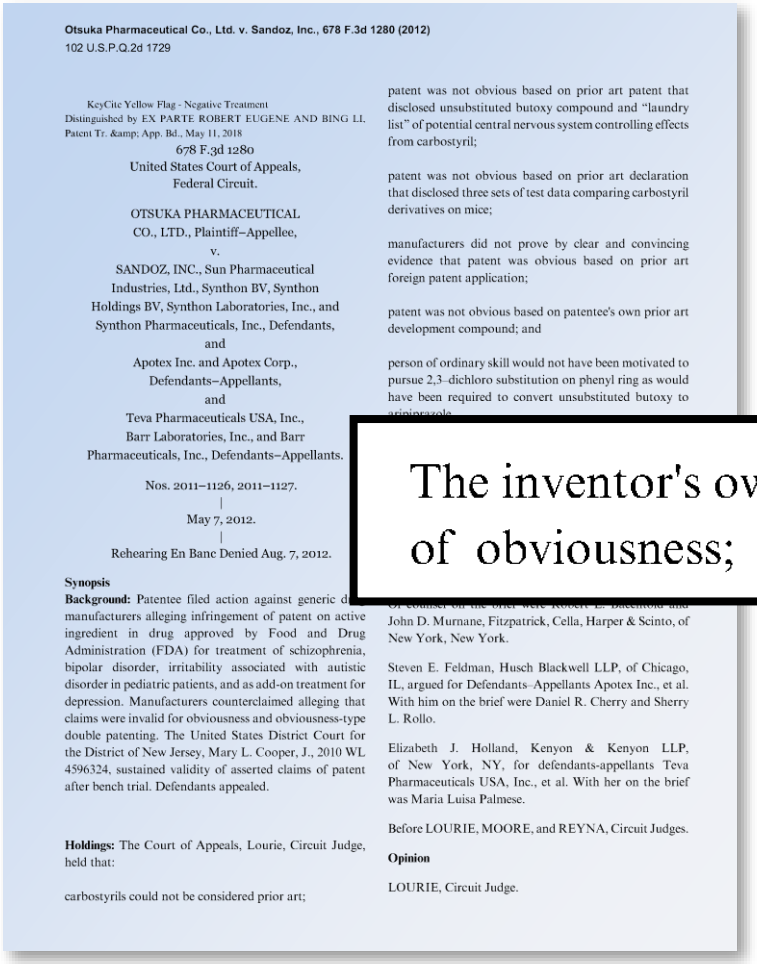
Floyd Romesberg, Ph.D.
Deposition

A. Let me answer the question that I think you're getting at is that I think that we would need -- you would need for Tsien's sequencing by synthesis method, I think the answer is applicable to that, that you would need something greater than 95 percent for cleavage of the 3' hydrox group, if that's the question that you're asking.

Ex. 2126, Romesberg Dep., 70:12-18

IPR2018-00291, PO Response, 35-36

Illumina Improperly Relies on Hindsight



Otsuka Pharmaceutical Co., Ltd. v. Sandoz, Inc., 678 F.3d 1280 (2012)
102 U.S.P.Q.2d 1729

KeyCite Yellow Flag - Negative Treatment
Distinguished by EX PARTE ROBERT EUGENE AND BING LI, Patent Tr. & App. Bd., May 11, 2018

678 F.3d 1280
United States Court of Appeals,
Federal Circuit.

OTSUKA PHARMACEUTICAL CO., LTD., Plaintiff–Appellee,
v.
SANDOZ, INC., Sun Pharmaceutical Industries, Ltd., Synthon BV, Synthon Holdings BV, Synthon Laboratories, Inc., and Synthon Pharmaceuticals, Inc., Defendants,
and
Apotex Inc. and Apotex Corp., Defendants–Appellants,
and
Teva Pharmaceuticals USA, Inc., Barr Laboratories, Inc., and Barr Pharmaceuticals, Inc., Defendants–Appellants.

Nos. 2011–1126, 2011–1127.
|
May 7, 2012.
|
Rehearing En Banc Denied Aug. 7, 2012.

Synopsis
Background: Patentee filed action against generic d… manufacturers alleging infringement of patent on active ingredient in drug approved by Food and Drug Administration (FDA) for treatment of schizophrenia, bipolar disorder, irritability associated with autistic disorder in pediatric patients, and as add-on treatment for depression. Manufacturers counterclaimed alleging that claims were invalid for obviousness and obviousness-type double patenting. The United States District Court for the District of New Jersey, Mary L. Cooper, J., 2010 WL 4596324, sustained validity of asserted claims of patent after bench trial. Defendants appealed.

Holdings: The Court of Appeals, Lourie, Circuit Judge, held that:

carbostyrils could not be considered prior art;

patent was not obvious based on prior art patent that disclosed unsubstituted butoxy compound and "laundry list" of potential central nervous system controlling effects from carbostyril;

patent was not obvious based on prior art declaration that disclosed three sets of test data comparing carbostyril derivatives on mice;

manufacturers did not prove by clear and convincing evidence that patent was obvious based on prior art foreign patent application;

patent was not obvious based on patentee's own prior art development compound; and

person of ordinary skill would not have been motivated to pursue 2,3–dichloro substitution on phenyl ring as would have been required to convert unsubstituted butoxy to aripiprazole.

…Of counsel on the brief were Robert L. Baechtold and John D. Murnane, Fitzpatrick, Cella, Harper & Scinto, of New York, New York.

Steven E. Feldman, Husch Blackwell LLP, of Chicago, IL, argued for Defendants–Appellants Apotex Inc., et al. With him on the brief were Daniel R. Cherry and Sherry L. Rollo.

Elizabeth J. Holland, Kenyon & Kenyon LLP, of New York, NY, for defendants-appellants Teva Pharmaceuticals USA, Inc., et al. With her on the brief was Maria Luisa Palmese.

Before LOURIE, MOORE, and REYNA, Circuit Judges.

Opinion

LOURIE, Circuit Judge.

> The inventor's own path itself never leads to a conclusion of obviousness; that is hindsight. What matters is the

Otsuka Pharm. Co. v. Sandoz, Inc.,
678 F.3d 1280, 1296 (Fed. Cir. 2012)

IPR2018-00291, PO Response, 47

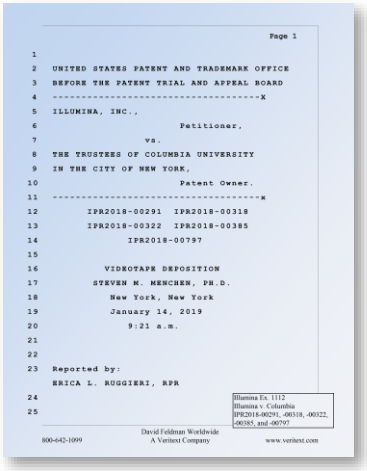
Dr. Menchen's Sanger Sequencing Patents Are Irrelevant

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

Dr. Menchen Did Not Include "Allyloxy" in His Sanger Patents Because of Metzker 1994



Page 1

UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,
vs.
THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK,
Patent Owner.

IPR2018-00291 IPR2018-00318
IPR2018-00322 IPR2018-00385
IPR2018-00797

VIDEOTAPE DEPOSITION
STEVEN M. MENCHEN, PH.D.
New York, New York
January 14, 2019
9:21 a.m.

Reported by:
ERICA L. RUGGIERI, RPR

Illumina Ex. 1112
Illumina v. Columbia
IPR2018-00291, -00318, -00322, -00385, and -00797

David Feldman Worldwide
A Veritext Company
800-642-1099
www.veritext.com

Stephen Menchen, Ph.D.
Deposition

Q. What would a person of ordinary skill in the art in 2000 gather from Metzker 1994 in terms of the incorporation efficiency of allyl groups, do you know?

A. They would look at Metzker and see that when the allyl group's on dA that it's inefficiently incorporated with Vent.

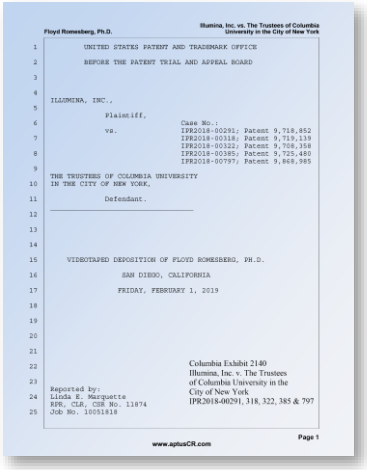
Q. How come you still identified allyl groups as a suitable modifier for 3' position in your patents?

A. Because that's what Metzker calls it. But he definitely refers to the structure of propenyl in that paper. He calls it allyl in the paper.

Ex. 1112, Menchen Dep., 188:20-189:13

IPR2018-00291, Sur-Reply, 7-8

Illumina's Dependence on Sanger Results Is Misplaced Because Sanger Is Fundamentally Different Than SBS


Floyd Romesberg, Ph.D. — Illumina, Inc. vs. The Trustees of Columbia University in the City of New York

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,

Plaintiff,

vs.

Case No.:
IPR2018-00291; Patent 9,718,852
IPR2018-00318; Patent 9,719,139
IPR2018-00322; Patent 9,708,358
IPR2018-00385; Patent 9,725,480
IPR2018-00797; Patent 9,868,985

THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK,

Defendant.

VIDEOTAPED DEPOSITION OF FLOYD ROMESBERG, PH.D.

SAN DIEGO, CALIFORNIA

FRIDAY, FEBRUARY 1, 2019

Columbia Exhibit 2140
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291, 318, 322, 385 & 797

Reported by:
Linda E. Marquette
RPR, CLR, CSR No. 11874
Job No. 10051810

www.aptusCR.com

Page 1

Floyd Romesberg, Ph.D.
Deposition

Q. …Well, we're just talking about terminating the DNA strands. We agree that for Sanger sequencing, you can't terminate all of the strands because you wouldn't be able to generate a ladder, correct?

A. They have different desires for the extent of termination. It just – it's even weird to talk about calling them termination in this way in Sanger sequencing compared to sequencing by synthesis. But, okay. It's not the way I think about it. I think it more as incorporation. But, yeah, they are blocks, so they are terminating, sure.

Q. Okay. But for SBS the goal would be to have very high termination, almost complete termination of the growing DNA strands to prevent out-of-phase sequencing, correct?

A. Sure.

Ex. 2140, Romesberg Dep., 149:1-17

IPR2018-00291, Sur-Reply, 6-7

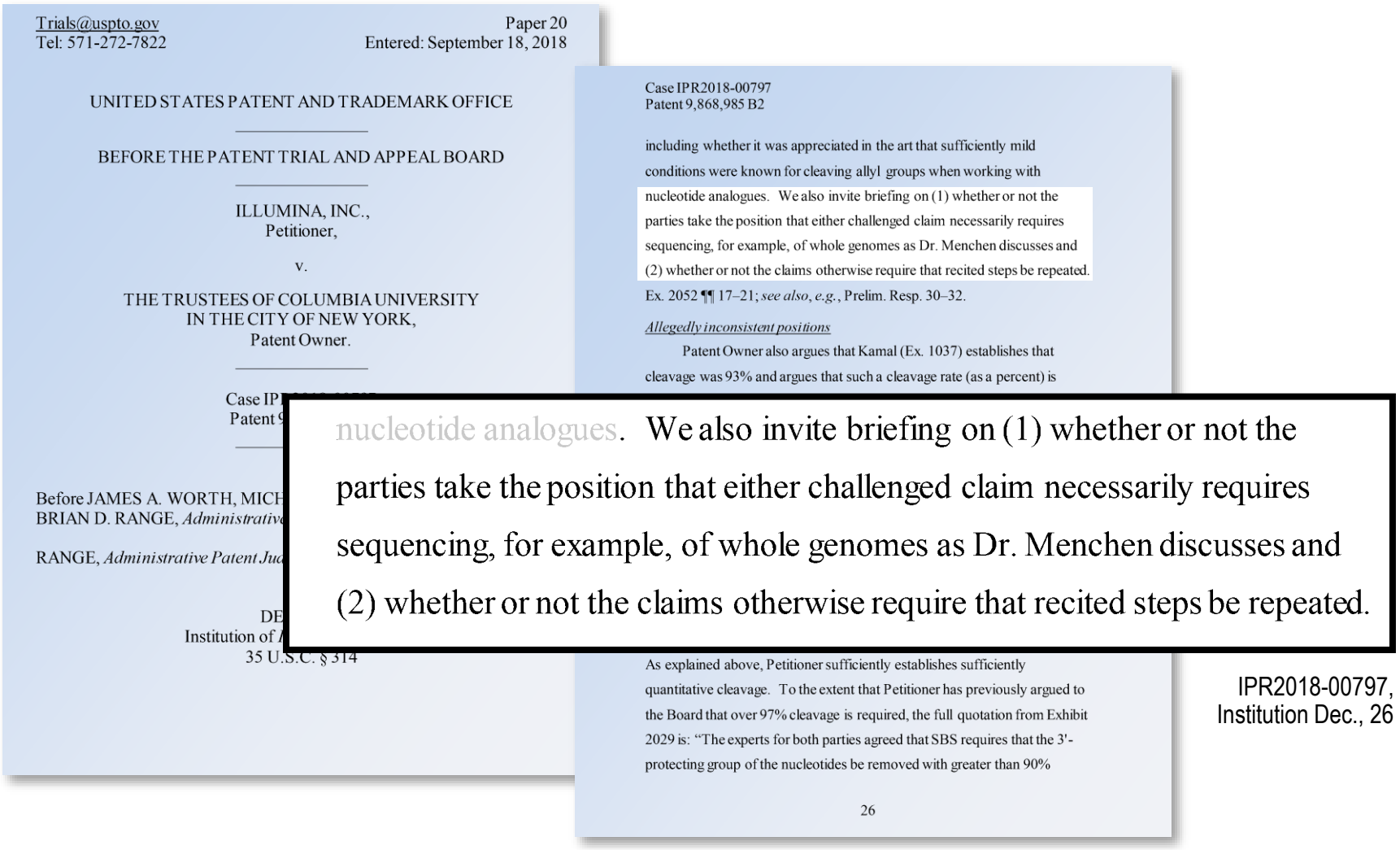
SBS Methods Require Multiple Cycles

Relevant to:

IPR2018-00291, 318, 322, 385: Grounds 1-2

IPR2018-00797: Grounds 1-3

The Board Invited Briefing On Whether Columbia's Method Patent Requires Multiple Cycles of SBS: It Does



Trials@uspto.gov
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Paper 20
Entered: September 18, 2018

UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

ILLUMINA, INC.,
Petitioner,

v.

THE TRUSTEES OF COLUMBIA UNIVERSITY
IN THE CITY OF NEW YORK,
Patent Owner.

Case IP
Patent 9

Before JAMES A. WORTH, MICH
BRIAN D. RANGE, *Administrativ*

RANGE, *Administrative Patent Ju*

DE
Institution of
35 U.S.C. § 314

Case IPR2018-00797
Patent 9,868,985 B2

including whether it was appreciated in the art that sufficiently mild conditions were known for cleaving allyl groups when working with nucleotide analogues. We also invite briefing on (1) whether or not the parties take the position that either challenged claim necessarily requires sequencing, for example, of whole genomes as Dr. Menchen discusses and (2) whether or not the claims otherwise require that recited steps be repeated. Ex. 2052 ¶¶ 17–21; *see also*, *e.g.*, Prelim. Resp. 30–32.

Allegedly inconsistent positions

Patent Owner also argues that Kamal (Ex. 1037) establishes that cleavage was 93% and argues that such a cleavage rate (as a percent) is

As explained above, Petitioner sufficiently establishes sufficiently quantitative cleavage. To the extent that Petitioner has previously argued to the Board that over 97% cleavage is required, the full quotation from Exhibit 2029 is: "The experts for both parties agreed that SBS requires that the 3'-protecting group of the nucleotides be removed with greater than 90%

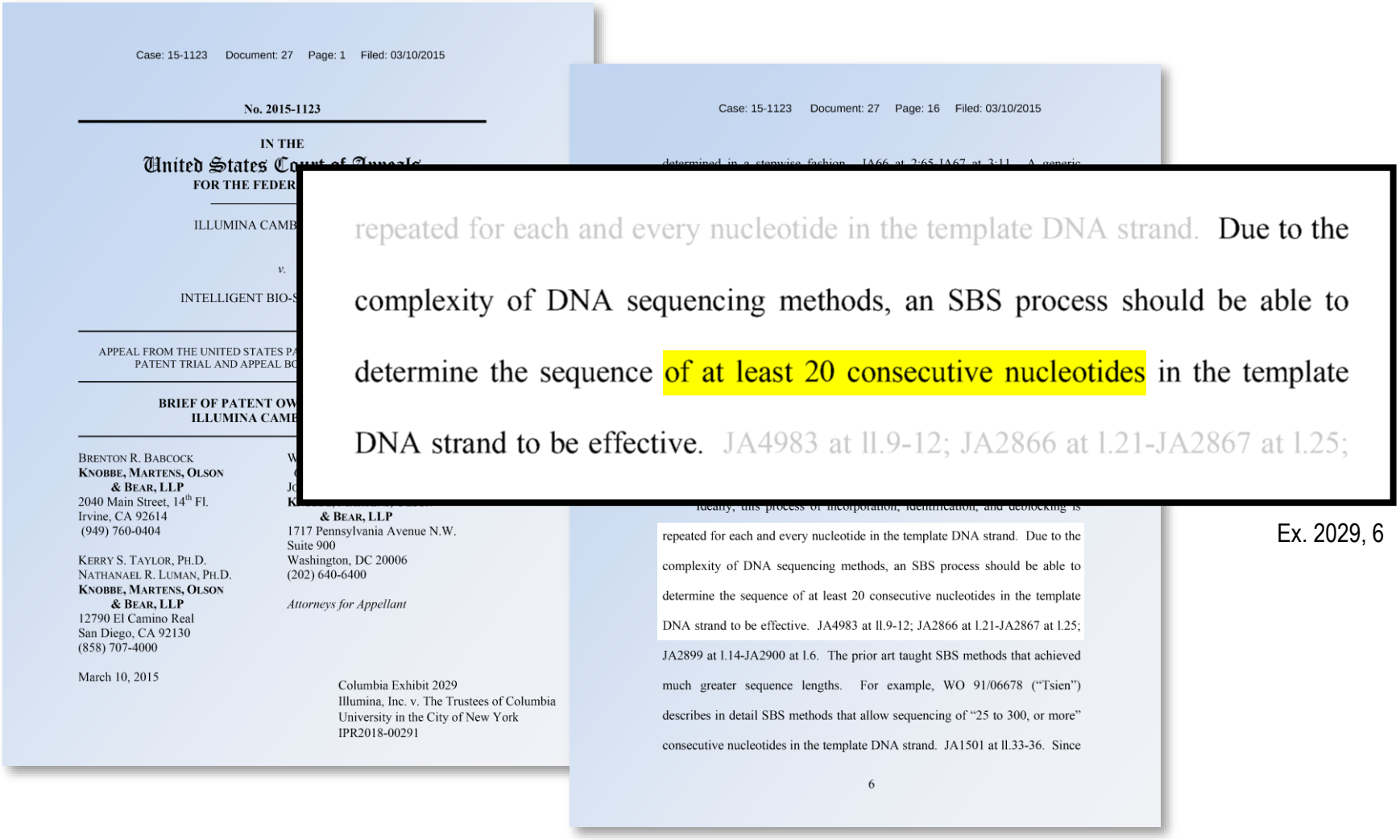
26

nucleotide analogues. We also invite briefing on (1) whether or not the parties take the position that either challenged claim necessarily requires sequencing, for example, of whole genomes as Dr. Menchen discusses and (2) whether or not the claims otherwise require that recited steps be repeated.

IPR2018-00797, Institution Dec., 26

IPR2018-00797, PO Response, 48

Illumina Previously Conceded That SBS Should Be Able to Sequence 20 Bases



Case: 15-1123 Document: 27 Page: 1 Filed: 03/10/2015

No. 2015-1123

IN THE

United States Court of Appeals

FOR THE FEDER

ILLUMINA CAMB

v.

INTELLIGENT BIO-S

APPEAL FROM THE UNITED STATES PA
PATENT TRIAL AND APPEAL BO

BRIEF OF PATENT OW
ILLUMINA CAME

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Attorneys for Appellant

March 10, 2015

Columbia Exhibit 2029
Illumina, Inc. v. The Trustees of Columbia University in the City of New York
IPR2018-00291

Case: 15-1123 Document: 27 Page: 16 Filed: 03/10/2015

repeated for each and every nucleotide in the template DNA strand. Due to the complexity of DNA sequencing methods, an SBS process should be able to determine the sequence of at least 20 consecutive nucleotides in the template DNA strand to be effective. JA4983 at ll.9-12; JA2866 at l.21-JA2867 at l.25; JA2899 at l.14-JA2900 at l.6. The prior art taught SBS methods that achieved much greater sequence lengths. For example, WO 91/06678 ("Tsien") describes in detail SBS methods that allow sequencing of "25 to 300, or more" consecutive nucleotides in the template DNA strand. JA1501 at ll.33-36. Since

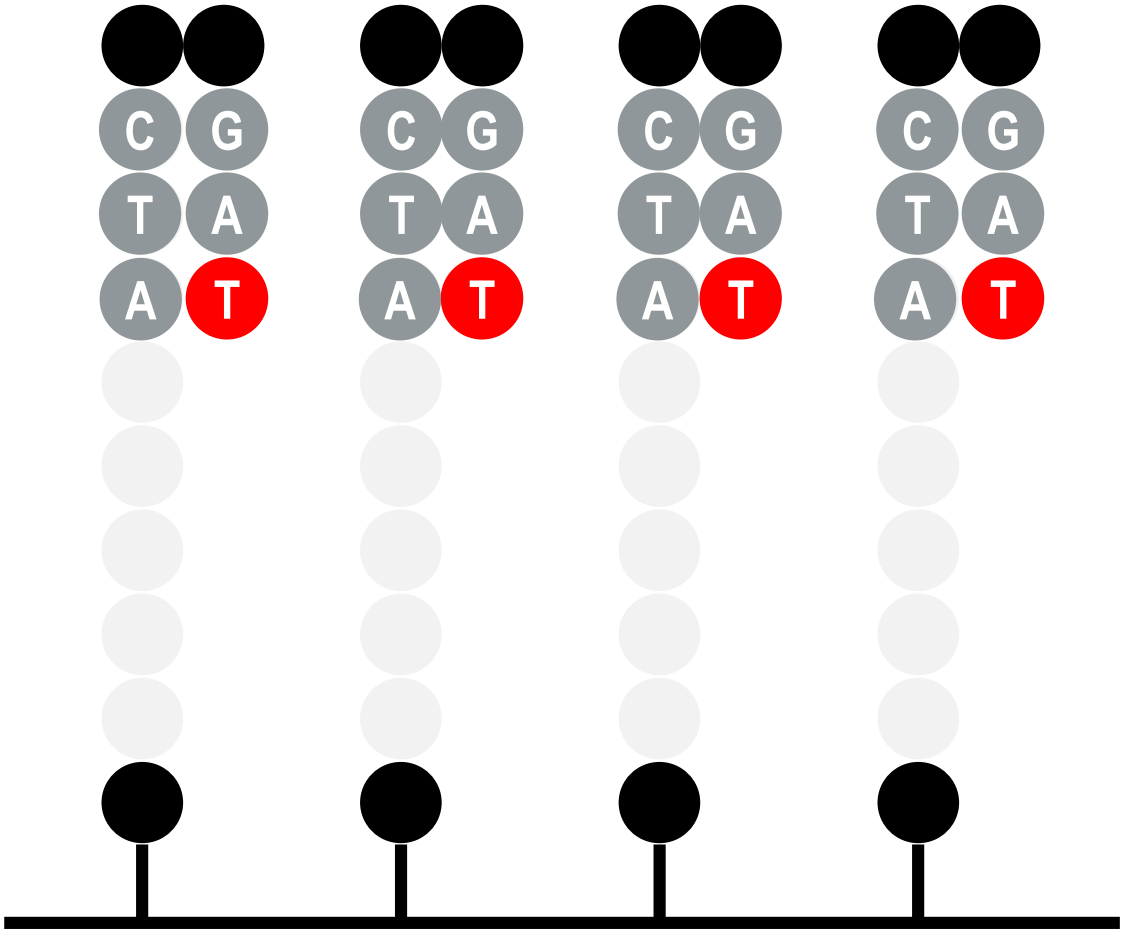
6

repeated for each and every nucleotide in the template DNA strand. Due to the complexity of DNA sequencing methods, an SBS process should be able to determine the sequence of at least 20 consecutive nucleotides in the template DNA strand to be effective. JA4983 at ll.9-12; JA2866 at l.21-JA2867 at l.25;

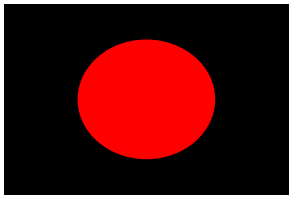
Ex. 2029, 6

IPR2018-00797, PO Response, 49

Efficient Incorporation



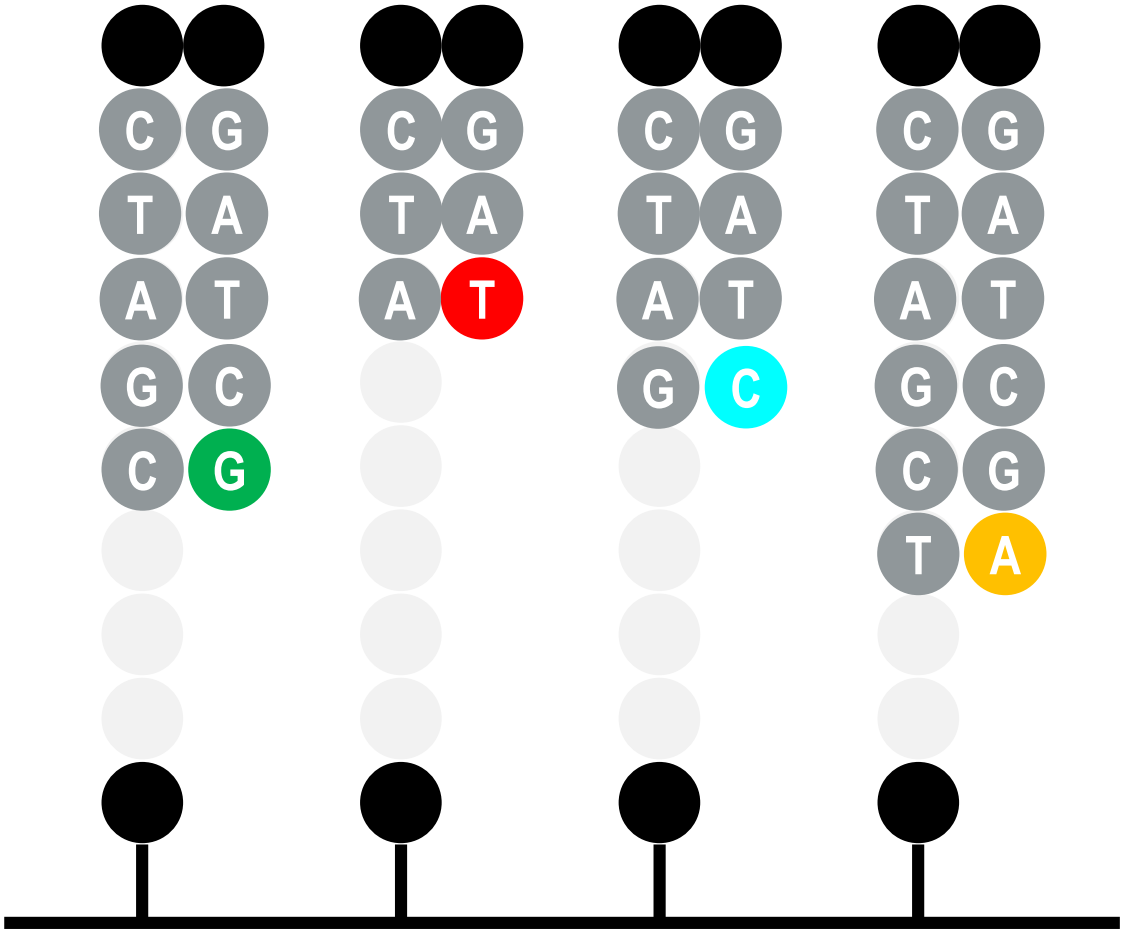
Label Detection:



Incorporated Base = T

IPR2018-00291, PO Response, 5-6; Sur-Reply, 6-7, 9, 26-27; Ex. 2126, 76:24-77:10; Ex. 2140, 149:13-17; IPR2018-00797, Institution Decision, 22

Out of Phase



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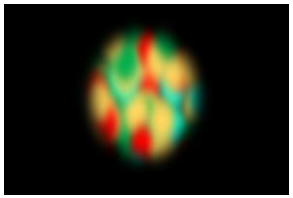


Incorporated Base = ?

IPR2018-00291, PO Response, 5-6; Sur-Reply, 6-7, 9, 26-27; Ex. 2126, 76:24-77:10; Ex. 2140, 149:13-17; IPR2018-00797, Institution Decision, 22

ADDITIONAL SLIDES

A POSA Would Consult Welch 1999, Which Is Directed to SBS (BASS) and Does Not Teach Using Small Capping Groups (max 3.7Å)

FULL PAPER

Syntheses of Nucleosides Designed for Combinatorial DNA Sequencing

Mike B. Welch,[a] Carlos I. Martinez,[b] Alex J. Zhang,[a] Song Jin,[a] Richard Gibbs,[b] and Kevin Burgess*[a]

Abstract: Nucleoside triphosphates **I** with 3′-O-blocking groups that are both photolabile and fluorescent were required to investigate the viability of a strategy for sequencing DNA in a combinatorial fashion (see Figure 1). Four compounds were prepared to realize this goal. Two of the dansyl-functional benzyl) ether gr two, **18a** and **18** carbonate groups tion of these anal

by using five different DNA replicating enzymes, but the analogues were not incorporated. These results were surprising in view of the fact that previous studies had shown that 3′-*O*-(2″-nitrobenzyl)adenosine triphosphate **II** was incorporated by *Bst* DNA polymerase

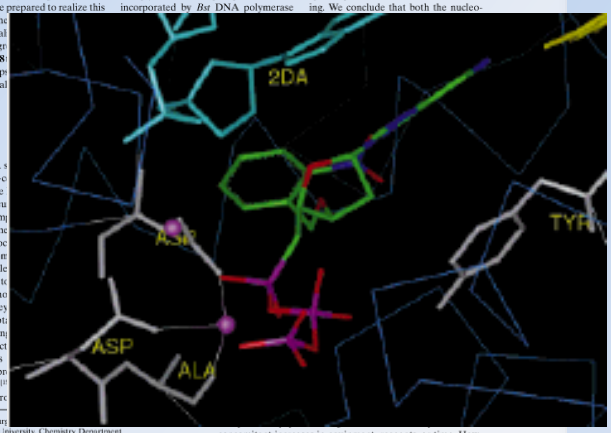
crystal structure as a model demonstrates that analogues **14a**, **14t**, **18a** and **18t** are too large to fit into the enzyme active site, whereas accommodation of the unsubstituted 2-nitrobenzyl compound **II** is much less demanding. We conclude that both the nucleo-

Complete DNA s costly and time-c sequencing large Most of the cu technological imp Gilbert[3] scheme systems for proc cleotides on com chines[5, 6] couple electrophoresis t trophoresis is no nevertheless they factors to accept tional sequencin require gel elect hexamer strings wherein the appr cleotide library[1] remains to be pr

[a] Prof. Dr. K. Bur Texas A & M University, Chemistry Department P. O. Box 300012

[b] C. I. Martinez, R Department of M Baylor College o Houston, TX 770

Chem. Eur. J. **1999**, *5*,



T7 Polymerase/Primer-Template/ 3'-O-modified dNTP

FULL PAPER K. Burgess et al.

Conclusions

3′-*O*-Blocked nucleoside triphosphates, wherein the 3′-protecting group is both photolabile and fluorescent, are synthetically accessible. However, they tend to be too big to fit into the active site of DNA polymerases as evidenced by the data from the activity screens and the molecular-simulation experiments. It seems clear that modifications to the polymerase enzyme as well as to the nucleoside triphosphate are required if BASS is to be developed into a viable sequencing scheme. There are indications that modern methods for combinatorial mutagenesis of proteins, gene shuffling,[48] have the potential to overcome this obstacle, but those methods must be refined further before this goal can be realized.

Experimental Section

General procedures: High-field NMR spectra were recorded on a Unity+ 300 spectrometer (^{1}H at 300 MHz, ^{13}C at 75 MHz). ^{1}H chemical shifts are reported in δ relative to $CHCl_3$ (7.23 ppm) as internal standard, and ^{13}C chemical shifts are reported in ppm relative to $CDCl_3$ (77.0 ppm) unless otherwise specified. Multiplicities in ^{1}H NMR are reported as (br) broad, (s) singlet, (d) doublet, (t) triplet, (q) quartet, (p) quintet, and (m) multiplet.

J = 10.5, 1.5 Hz, 1H), 4.78 (br s, 1H), 4.55–4.50 (m, 3H), 4.30 (t, J = 6.3 Hz, 2H), 4.00–3.80 (m, 3H), 3.16 (q, J = 6.6 Hz, 2H), 2.35 (ddd, J = 13.2, 5.7, 2.4 Hz, 1H), 2.22–2.12 (m, 1H), 1.75 (p, J = 7.2 Hz, 2H), 1.63 (s, 3H), 1.59–1.38 (m, 4H), 1.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.3, 163.2, 156.3, 150.7, 148.8, 136.9, 135.5, 135.2, 134.1, 133.8, 132.8, 132.2, 130.5, 130.2, 130.1, 128.4, 128.0, 127.9, 125.9, 1176, 110.3, 87.0, 85.4, 72.0, 65.5, 64.0, 41.6, 41.1, 40.8, 29.6, 28.2, 27.0, 23.1, 19.3, 12.8; HRMS (positive-ion FAB, nitrobenzyl alcohol (NBA)): calcd for $C_{41}H_{50}N_4O_{10}SiNa$ 851.3299, found 851.3327.

***3′-O*-[4″-(*N*-Allyloxycarbonyl)-5″-aminopent-1-yl-oxycarbonyl)-2″-nitrophenylmethyl]thymidine (4)**: Compound **3** (700 mg, 0.84 mmol), THF (8 mL), and terabutylammonium fluoride (TBAF) (1.26 mL, 1.26 mmol) were stirred at 25 °C for 15 min. The reaction was concentrated and purified directly by flash chromatography with a gradient of 100% EtOAc, then 1 to 5% MeOH in EtOAc as the eluant to give **4** as a foam (589 mg, 88% yield). R_f = 0.11 (75% EtOAc/hexanes); IR (neat): $\tilde{\nu}$ = 3479, 2947, 1720, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 8.54 (d, J = 1.8 Hz, 1H), 8.06 (dd, J = 8.1, 1.5 Hz, 1H), 7.60 (s, 1H), 7.24 (d, J = 8.1 Hz, 1H), 6.17 (t, J = 6.6 Hz, 1H), 5.89–5.76 (m, 1H), 5.44 (s, 2H), 5.21 (d, J = 17.4 Hz, 1H), 5.12 (d, J = 10.5 Hz, 1H), 4.97 (br, 1H), 4.47–4.41 (m, 3H), 4.28 (t, J = 6.3 Hz, 2H), 4.00 (t, J = 6.6 Hz, 2H), 3.89 (br, 1H), 3.81–3.69 (m, 2H), 3.12 (q, J = 6.6 Hz, 2H), 3.95 (br, 1H), 2.25–2.19 (m, 2H), 1.99 (s, 3H), 1.74 (p, J = 7.5 Hz, 2H), 1.58–1.35 (m, 4H); ^{13}C NMR ($CDCl_3$, 75 MHz): δ = 164.3, 163.2, 156.4, 150.7, 148.7, 136.8, 135.5, 133.9, 132.8, 130.5, 128.4, 125.8, 1175, 110.1, 87.0, 86.5, 71.1, 65.6, 65.4, 62.1, 41.5, 40.7, 40.3, 29.5, 28.2, 23.0, 13.1; HRMS (positive-ion FAB, NBA): calcd for $C_{32}H_{38}N_6O_{11}Na$ 613.2122, found 613.2122.

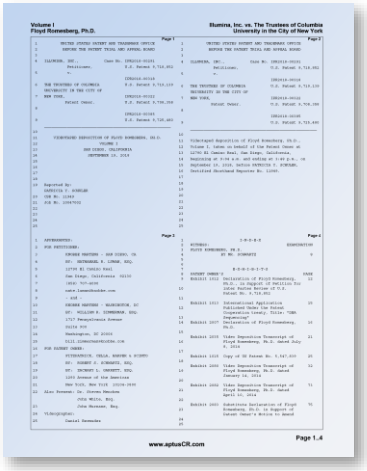
***3′-O*-[4″-(*N*-Allyloxycarbonyl)-5″-aminopent-1-yl-oxycarbonyl)-2″-nitro-**

Conclusions

3′-O-Blocked nucleoside triphosphates, wherein the 3′-protecting group is both photolabile and fluorescent, are synthetically accessible. However, they tend to be too big to fit into the active site of DNA polymerases as evidenced by the data from the activity screens and the molecular-simulation experiments. It seems clear that modifications to the polymerase enzyme as well as to the nucleoside triphosphate are required if BASS is to be developed into a viable sequencing scheme. There are indications that modern methods for combinatorial mutagenesis of proteins, gene shuffling,[48] have the potential to overcome this obstacle, but those methods must be refined further before this goal can be realized.

Ex. 1023, 956

No Expectation That Small Capping Group Met Tsien's Requirements for SBS



Floyd Romesberg, Ph.D.
Deposition

Q. …It is your opinion that a person of ordinary skill in the art would not have had an expectation that a small and removable protecting group would also possess all of the listed properties of Tsien's protecting group?

A. Yes.

Ex. 2126, Romesberg Dep., 82:21-83:2

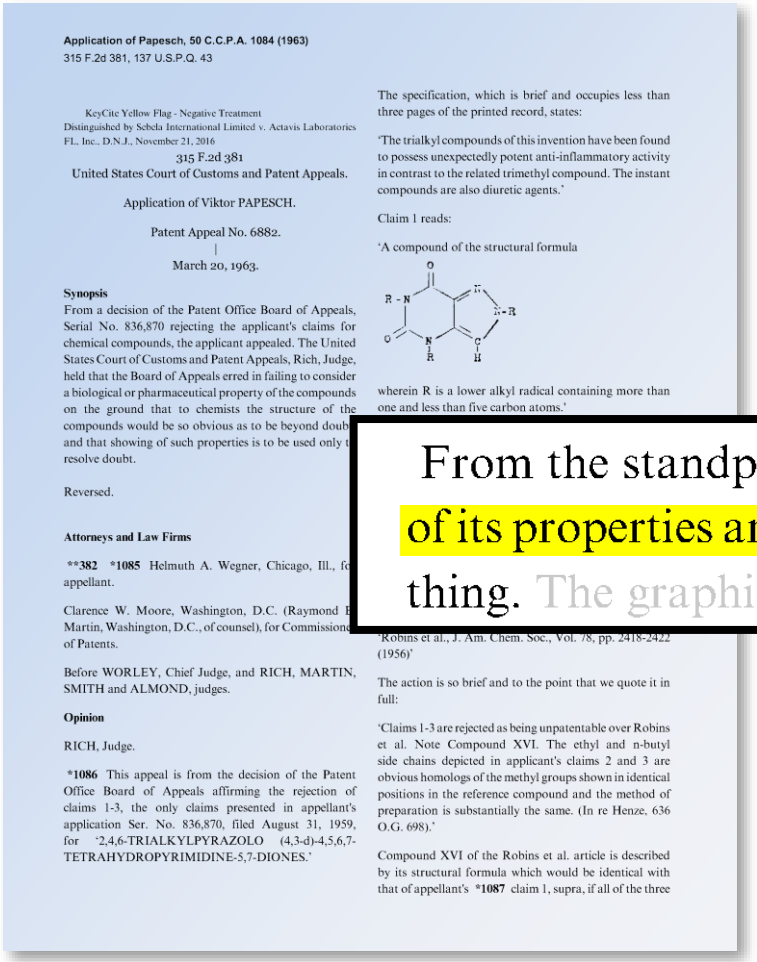
IPR2018-00291, PO Response, 47

No Expectation a 3'-O-Allyl Thymine, Cytosine or Guanine Nucleotide Would be Incorporated

	AMV-RT	M-MuLV-RT	*Bst*	AmpliTaq	Vent(exo-)	*rTth*
3'-O-methyl-dATP	✓	✓	✗	✗	✗	✗
3'-O-methyl-dTTP	✗	✗	✓	✓	✓	✓

IPR2018-00291, PO Response, 48-50

A Compound and All of Its Properties Are Inseparable



Application of Papesch, 50 C.C.P.A. 1084 (1963)
315 F.2d 381, 137 U.S.P.Q. 43

KeyCite Yellow Flag - Negative Treatment
Distinguished by Sebela International Limited v. Actavis Laboratories FL, Inc., D.N.J., November 21, 2016

315 F.2d 381
United States Court of Customs and Patent Appeals.

Application of Viktor PAPESCH.

Patent Appeal No. 6882.

March 20, 1963.

Synopsis
From a decision of the Patent Office Board of Appeals, Serial No. 836,870 rejecting the applicant's claims for chemical compounds, the applicant appealed. The United States Court of Customs and Patent Appeals, Rich, Judge, held that the Board of Appeals erred in failing to consider a biological or pharmaceutical property of the compounds on the ground that to chemists the structure of the compounds would be so obvious as to be beyond doubt and that showing of such properties is to be used only to resolve doubt.

Reversed.

Attorneys and Law Firms

**382 *1085 Helmuth A. Wegner, Chicago, Ill., for appellant.

Clarence W. Moore, Washington, D.C. (Raymond E. Martin, Washington, D.C., of counsel), for Commissioner of Patents.

Before WORLEY, Chief Judge, and RICH, MARTIN, SMITH and ALMOND, judges.

Opinion

RICH, Judge.

*1086 This appeal is from the decision of the Patent Office Board of Appeals affirming the rejection of claims 1-3, the only claims presented in appellant's application Ser. No. 836,870, filed August 31, 1959, for '2,4,6-TRIALKYLPYRAZOLO (4,3-d)-4,5,6,7-TETRAHYDROPYRIMIDINE-5,7-DIONES.'

The specification, which is brief and occupies less than three pages of the printed record, states:

'The trialkyl compounds of this invention have been found to possess unexpectedly potent anti-inflammatory activity in contrast to the related trimethyl compound. The instant compounds are also diuretic agents.'

Claim 1 reads:

'A compound of the structural formula

wherein R is a lower alkyl radical containing more than one and less than five carbon atoms.'

'Robins et al., J. Am. Chem. Soc., Vol. 78, pp. 2418-2422 (1956)'

The action is so brief and to the point that we quote it in full:

'Claims 1-3 are rejected as being unpatentable over Robins et al. Note Compound XVI. The ethyl and n-butyl side chains depicted in applicant's claims 2 and 3 are obvious homologs of the methyl groups shown in identical positions in the reference compound and the method of preparation is substantially the same. (In re Henze, 636 O.G. 698).'

Compound XVI of the Robins et al. article is described by its structural formula which would be identical with that of appellant's *1087 claim 1, supra, if all of the three

From the standpoint of patent law, a compound and all of its properties are inseparable; they are one and the same thing. The graphic formulae, the chemical nomenclature,

In re Papesch, 315 F.2d 381, 391 (C.C.P.A. 1963)