

008/289221

Class	Subclass

ISSUE CLASSIFICATION



94

UTILITY SERIAL NUMBER 008/289221	PATENT DATE	PATENT NUMBER
-------------------------------------	-------------	---------------

SERIAL NUMBER 008/289221	FILING DATE 08/02/94	CLASS 289	SUBCLASS	GROUP ART UNIT 1804	EXAMINER Rees
-----------------------------	-------------------------	--------------	----------	------------------------	------------------

APPLICANTS

MARK H. SKOLNICK, SALT LAKE CITY, UT; DAVID E. GOLDMAN, SALT LAKE CITY, UT; YOUNG & ALI, SALT LAKE CITY, UT; JEFF JOHNSON, SALT LAKE CITY, UT; ALEXANDER HARRIS, SALT LAKE CITY, UT; KEITH D. HARRISON, SALT LAKE CITY, UT; CONNIE H. SHATTUCK-EDWARDS, SALT LAKE CITY, UT; SEAN V. TAVTIGIAN, SALT LAKE CITY, UT; ROGER W. WISEMAN, DURHAM, NC; P. ANDREW FUTREAL, DURHAM, NC.

72608

THIS APPLICATION IS A C/P OF

DR ✓ 9/10/94

ABANDONED

VERIFIED

NONE DR 9/10/94

FOREIGN FILING LICENSE GRANTED 07/09/94

Foreign priority claimed 35 USC 119 conditions met	<input type="checkbox"/> yes <input checked="" type="checkbox"/> no <input type="checkbox"/> yes <input checked="" type="checkbox"/> no	AS FILED →	STATE OR COUNTRY UT	SHEETS DRWGS. 4	TOTAL CLAIMS 71	INDEP. CLAIMS 19	FILING FEE RECEIVED \$3,198.00	ATTORNEY'S DOCKET NO. 248041003471
Verified and Acknowledged		Examiner's Initials						

ADDRESS

VERONICA DELEON HOWARD & DEVALETTE
1301 NEW YORK AVENUE NW
SUITE 1000
WASHINGTON DC 20005

TITLE

GENE EXPRESSION AND CANCER SUSCEPTIBILITY GENE

U.S. DEPT. OF COMM./PAT. & TM—PTO-436L (Rev.12-94)

PARTS OF APPLICATION FILED SEPARATELY				Applications Examiner			
NOTICE OF ALLOWANCE MAILED		Assistant Examiner		CLAIMS ALLOWED			
				Total Claims		Print Claim	
ISSUE FEE		Primary Examiner		DRAWING			
Amount Due	Date Paid			Sheets Drwg.		Figs. Drwg.	
Label Area		PREPARED FOR ISSUE		ISSUE BATCH NUMBER			
		WARNING: The information disclosed herein may be restricted. Unauthorized disclosure may be prohibited by the United States Code Title 35, Sections 122, 181 and 368. Possession outside the U.S. Patent & Trademark Office is restricted to authorized employees and contractors only.					

briefed group 1560

PATENT APPLICATION



08289221

APPROVED FOR LICENSE

AUG 16 94 26

INITIALS



08/08/289221

Date
Entered
or
Counted

CONTENTS

Date
Received
or
Mailed

ABANDONED

Handwritten signature/initials

1. Application _____ papers.
2. *Raw Sequence Listing (OK)* 8/19/94
3. *Ltr Re: doc & fees* 7/26/94
4. *Petition 1.48 w/ext. (4 MAS)* 2-13-95
5. *Requirement For Information* 4-18-95
6. *Response* 8-15-95
7. *Response 1.48* 10-26-95
8. *Ref. (C. 10/1/94)* 3/11/96
9. *ALPHABETIC AND* 9-11-96
10. *Notice of Abn* 9-19-96
11. *Express Aband* 9-10-96
12. *Request for Access* 12-8-97
13. *Request for Access* 1-8-98
14. *Request for Access* 1-20-98
15. *Request for Access* 4/30/03
16. _____
17. _____
18. _____
19. _____
20. _____
21. _____
22. _____
23. _____
24. _____
25. _____
26. _____
27. _____
28. _____
29. _____
30. _____
31. _____
32. _____

SEARCHED			
Class	Sub.	Date	Exmr.

SEARCH NOTES		
	Date	Exmr.

INTERFERENCE SEARCHED			
Class	Sub.	Date	Exmr.

Staple Issue Slip Here

POSITION		ID NO.	DATE
CLASSIFIER		8	8-16-94
EXAMINER		451	8/23/94
TYPIST		343	7/9/96
VERIFIER			
CORPS CORR.			
SPEC. HAND		308	5/22/94
FILE MAINT.		445	8/25/94
DRAFTING			

INDEX OF CLAIMS


Claim		Date			
Final	Original				
	1				
	2				
	3				
	4				
	5				
	6				
	7				
	8				
	9				
	10				
	11				
	12				
	13				
	14				
	15				
	16				
	17				
	18				
	19				
	20				
	21				
	22				
	23				
	24				
	25				
	26				
	27				
	28				
	29				
	30				
	31				
	32				
	33				
	34				
	35				
	36				
	37				
	38				
	39				
	40				
	41				
	42				
	43				
	44				
	45				
	46				
	47				
	48				
	49				
	50				

SYMBOLS
✓ Rejected
= Allowed
- (Through numeral) Canceled
+ Restricted
N Non-elected
I Interference
A Appeal
O Objected

Claim		Date			
Final	Original				
	51				
	52				
	53				
	54				
	55				
	56				
	57				
	58				
	59				
	60				
	61				
	62				
	63				
	64				
	65				
	66				
	67				
	68				
	69				
	70				
	71				
	72				
	73				
	74				
	75				
	76				
	77				
	78				
	79				
	80				
	81				
	82				
	83				
	84				
	85				
	86				
	87				
	88				
	89				
	90				
	91				
	92				
	93				
	94				
	95				
	96				
	97				
	98				
	99				
	100				

PATENT APPLICATION SERIAL NO. 08/289221

U.S. DEPARTMENT OF COMMERCE
PATENT AND TRADEMARK OFFICE
FEE RECORD SHEET

BAR CODE LABEL		U.S. PATENT APPLICATION			
					
SERIAL NUMBER		FILING DATE	CLASS	GROUP ART UNIT	
08/289,221		08/12/94	435	1804	
APPLICANT	MARK H. SKOLNICK, SALT LAKE CITY, UT; DAVID E. GOLDAR, SALT LAKE CITY, UT; YOSHIO MIKI, SALT LAKE CITY, UT; JEFF SWENSON, SALT LAKE CITY, UT; ALEXANDER KAMB, SALT LAKE CITY, UT; KEITH D. HARSHMAN, SALT LAKE CITY, UT; DONNA M. SHATTUCK-EIDENS, SALT LAKE CITY, UT; SEAN V. TAVTIGIAN, SALT LAKE CITY, UT; ROGER W. WISEMAN, DURHAM, NC; P. ANDREW FUTREAL, DURHAM, NC.				
	CONTINUING DATA*** VERIFIED THIS APPLN IS A CIP OF 08/246,602 05/20/94 ABN _____				
	FOREIGN/PCT APPLICATIONS*** VERIFIED _____				
	FOREIGN FILING LICENSE GRANTED 07/09/96				
STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
UT	4	71	19	\$3,198.00	248841093471
ADDRESS	VENABLE BAETJER HOWARD & CIVILETTI 1201 NEW YORK AVENUE NW SUITE 1000 WASHINGTON DC 20005				
	TITLE 17Q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE				
This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above. By authority of the COMMISSIONER OF PATENTS AND TRADEMARKS					
Date		Certifying Officer			

08/289221

Commissioner of Patents & Trademarks
Washington, D.C. 20231

SAIR:G

Transmitted herewith for filing is the patent application of:

Inventor(s): Mark H. SKOLNICK, David E. GOLDGAR, Yoshio MIKI, Jeff SWENSON, Alexander KAMB, Keith D. HARSHMAN, Donna M. SHATTUCK-EIDENS and Sean V. TAVTIGIAN

For: 17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTABILITY GENE

Enclosed are:

- ☒ Four (4) sheet(s) of drawings, in triplicate.
☐ Assignment of the invention to _____
☐ Verified statement establishing small entity status under 37 CFR 1.9 and 37 CFR 1.27
☒ Statement Pursuant to 37 CFR §1.821(f) and Computer Disk.
☒ Application filed with NO DELCARATION and NO FEE pursuant to 37 CFR §1.37(d).

(COL. 1)		(COL. 2)	SMALL ENTITY		OTHER THAN SMALL ENTITY	
FOR:	NO. FILED	EXTRA	RATE	FEE		
BASIC FEE:				\$355.00	OR	\$710.00
TOTAL CLAIMS:	-20 =		X \$ 11	\$	OR	X \$ 22
INDEP CLAIMS:	- 3 =		X \$ 37	\$	OR	X \$ 74
MULTIPLE DEPENDENT CLAIM PRESENTED:			+ \$115	\$	OR	+ \$230
			TOTAL	\$	OR	TOTAL

- ☐ Charge Deposit Account No. 22-0261 in the amount of \$_____. Duplicate copies of this sheet are attached.
☐ A check in the amount of \$_____ to cover the filing fee and assignment recordation fee is attached.

Please direct all correspondence to: Jeffrey L. Ihnen

Respectfully submitted,

Jeffrey L. Ihnen
Jeffrey L. Ihnen
Registration No. 28,957
Attorney for Applicant

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., Suite 1000
Washington, D.C. 20005
202-962-4810

Dated: 12 August 1994

Atty. Docket No. 24884-109347-1



00/289221

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. (to be assigned)

Group Art Unit:

Filed: 12 August 1994

Examiner:

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTABILITY GENE

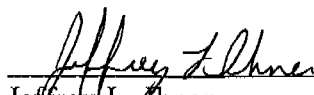
STATEMENT PURSUANT TO 37 CFR §1.821(f)

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In the matter of the above-identified application, filed concurrently herewith, Applicants present a computer readable copy of the Sequence Listing pursuant to 37 CFR §1.821(f). It is hereby certified that these copies are identical.

Respectfully submitted,



Jeffrey L. Ahnen
Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., Suite 1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347-1
Dated: 12 August 1994

02/289221

-115-

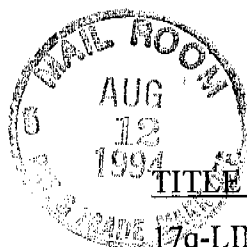
TITLE OF THE INVENTION

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

ABSTRACT OF THE DISCLOSURE

5 The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect a human breast and ovarian cancer predisposing gene (BRCA1), some mutant alleles of which cause susceptibility to cancer, in particular breast and ovarian cancer. More specifically, the invention relates to germline mutations in the BRCA1 gene and their use in the diagnosis of predisposition to breast and ovarian cancer. The present invention further relates to somatic mutations in the BRCA1 gene
10 in human breast and ovarian cancer and their use in the diagnosis and prognosis of human breast and ovarian cancer. Additionally, the invention relates to somatic mutations in the BRCA1 gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to the therapy of human cancers which have a mutation in the BRCA1 gene, including gene therapy, protein replacement therapy and protein mimetics. Finally, the
15 invention relates to the screening of drugs for cancer therapy.

08/289221



TITLE OF THE INVENTION

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

This work has been supported in part by National Cancer Institute grant Numbers CA-48711 and CA-55914. The United States government has certain rights in the invention.

5 CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of application Serial No. 08/246,602, filed on 20 May 1994, incorporated herein by reference.

FIELD OF THE INVENTION

10 The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect a human breast and ovarian cancer predisposing gene (BRCA1), some mutant alleles of which cause susceptibility to cancer, in particular, breast and ovarian cancer. More specifically, the invention relates to germline mutations in the BRCA1 gene and their use in the diagnosis of predisposition to breast and ovarian cancer. The present invention further relates to somatic mutations in the BRCA1 gene in human breast and
15 ovarian cancer and their use in the diagnosis and prognosis of human breast and ovarian cancer. Additionally, the invention relates to somatic mutations in the BRCA1 gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to the therapy of human cancers which have a mutation in the BRCA1 gene, including gene therapy, protein replacement therapy and protein mimetics. Finally, the invention relates to the screening of drugs
20 for cancer therapy.

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to provide additional details respecting the practice, are incorporated herein by reference, and for convenience, are referenced by author and date in the following text and respectively grouped in the appended bibliography.

BACKGROUND OF THE INVENTION

The genetics of cancer is complicated, involving multiple dominant, positive regulators of the transformed state (oncogenes) as well as multiple recessive, negative regulators (tumor suppressor genes). Over one hundred oncogenes have been characterized. Less than a dozen tumor suppressor genes have been identified, but the number is expected to increase beyond fifty (Knudson, 1993).

The involvement of so many genes underscores the complexity of the growth control mechanisms that operate in cells to maintain the integrity of normal tissue. This complexity is manifest in another way. So far, no single gene has been shown to participate in the development of all, or even the majority of human cancers. The most common oncogenic mutations are in the H-ras gene, found in 10-15% of all solid tumors (Anderson *et al.*, 1992). The most frequently mutated tumor suppressor genes are the TP53 gene, homozygously deleted in roughly 50% of all tumors, and CDKN2, which was homozygously deleted in 46% of tumor cell lines examined (Kamb *et al.*, 1994). Without a target that is common to all transformed cells, the dream of a "magic bullet" that can destroy or revert cancer cells while leaving normal tissue unharmed is improbable. The hope for a new generation of specifically targeted antitumor drugs may rest on the ability to identify tumor suppressor genes or oncogenes that play general roles in control of cell division.

The tumor suppressor genes which have been cloned and characterized influence susceptibility to: 1) retinoblastoma (RB1); 2) Wilms' tumor (WT1); 3) Li-Fraumeni (TP53); 4) Familial adenomatous polyposis (APC); 5) Neurofibromatosis type 1 (NF1); 6) Neurofibromatosis type 2 (NF2); 7) von Hippel-Lindau syndrome (VHL); 8) Multiple endocrine neoplasia type 2A (MEN2A); and 9) Melanoma (CDKN2).

Tumor suppressor loci that have been mapped genetically but not yet isolated include genes for: Multiple endocrine neoplasia type 1 (MEN1); Lynch cancer family syndrome 2 (LCFS2); Neuroblastoma (NB); Basal cell nevus syndrome (BCNS); Beckwith-Wiedemann syndrome (BWS); Renal cell carcinoma (RCC); Tuberous sclerosis 1 (TSC1); and Tuberous sclerosis 2 (TSC2). The tumor suppressor genes that have been characterized to date encode products with similarities to a variety of protein types, including DNA binding proteins (WT1), ancillary transcription regulators (RB1), GTPase activating proteins or GAPs (NF1), cytoskeletal components (NF2), membrane bound receptor kinases (MEN2A), cell cycle regulators (CDKN2) and others with no obvious similarity to known proteins (APC and VHL).

In many cases, the tumor suppressor gene originally identified through genetic studies has been shown to be lost or mutated in some sporadic tumors. This result suggests that regions of chromosomal aberration may signify the position of important tumor suppressor genes involved both in genetic predisposition to cancer and in sporadic cancer.

5 One of the hallmarks of several tumor suppressor genes characterized to date is that they are deleted at high frequency in certain tumor types. The deletions often involve loss of a single allele, a so-called loss of heterozygosity (LOH), but may also involve homozygous deletion of both alleles. For LOH, the remaining allele is presumed to be nonfunctional, either because of a preexisting inherited mutation, or because of a secondary sporadic mutation.

10 Breast cancer is one of the most urgent health issues for women today, as one in nine women can expect to develop breast cancer during her lifetime. Breast cancer is the most common cancer among women in the United States, with an annual incidence of 175,000 cases and 44,500 deaths. In view of the relatively high frequency of breast cancer, and because metastatic breast cancer is incurable, efforts to decrease breast cancer mortality have focused on early detection and improved
15 treatment.

Breast cancer has long been recognized to be, in part, a familial disease (Anderson, 1972). Numerous investigators have examined the evidence for genetic inheritance and concluded that the data are most consistent with dominant inheritance for a major susceptibility locus or loci. (Hill *et al.*, 1978; Bishop and Gardner, 1980; Go *et al.*, 1983; Willams and Anderson, 1984; Bishop *et al.*,
20 1988; Newman *et al.*, 1988; Claus *et al.*, 1991). Recent results demonstrate that at least three loci exist which convey susceptibility to breast cancer as well as other cancers. These loci are the TP53 locus on chromosome 17p (Malkin *et al.*, 1990), a 17q-linked susceptibility locus known as BRCA1 (Hall *et al.*, 1990), and one or more loci responsible for the unmapped residual. Hall *et al.* (1990) indicated that the inherited breast cancer susceptibility in kindreds with early age onset is linked to
25 chromosome 17q21; although subsequent studies by this group using a more appropriate genetic model partially refuted the limitation to early onset breast cancer (Margaritte *et al.*, 1992).

Most strategies for cloning the 17q-linked breast cancer predisposing gene (BRCA1) require precise genetic localization studies. The simplest model for the functional role of BRCA1 holds that alleles of BRCA1 that predispose to cancer are recessive to wild type alleles; that is, cells that contain
30 at least one wild type BRCA1 allele are not cancerous. However, cells that contain one wild type BRCA1 allele and one predisposing allele may occasionally suffer loss of the wild type allele either by random mutation or by chromosome loss during cell division (nondisjunction). All the progeny

of such a mutant cell lack the wild type function of BRCA1 and may develop into tumors. According to this model, predisposing alleles of BRCA1 are recessive, yet susceptibility to cancer is inherited in a dominant fashion: women who possess one predisposing allele (and one wild type allele) risk developing cancer, because their mammary epithelial cells may spontaneously lose the wild type BRCA1 allele. This model applies to a group of cancer susceptibility loci known as tumor suppressors or antioncogenes, a class of genes that includes the retinoblastoma gene and neurofibromatosis gene. By inference this model may also explain the BRCA1 function, as has recently been suggested (Smith *et al.*, 1992).

A second possibility is that BRCA1 predisposing alleles are truly dominant; that is, a wild type allele of BRCA1 cannot overcome the tumor forming role of the predisposing allele. Thus, a cell that carries both wild type and mutant alleles would not necessarily lose the wild type copy of BRCA1 before giving rise to malignant cells. Instead, mammary cells in predisposed individuals would undergo some other stochastic change(s) leading to cancer.

If BRCA1 predisposing alleles are recessive, the BRCA1 gene is expected to be expressed in normal mammary tissue but not functionally expressed in mammary tumors. In contrast, if BRCA1 predisposing alleles are dominant, the wild type BRCA1 gene may or may not be expressed in normal mammary tissue. However, the predisposing allele will likely be expressed in breast tumor cells.

The 17q linkage of BRCA1 was independently confirmed in three of five kindreds with both breast cancer and ovarian cancer (Narod *et al.*, 1991). These studies claimed to localize the gene within a very large region, 15 centimorgans (Cm), or approximately 15 million base pairs, to either side of the linked marker pCMM86 (D17S74). However, attempts to define the region further by genetic studies, using markers surrounding pCMMS6, proved unsuccessful. Subsequent studies indicated that the gene was considerably more proximal (Easton *et al.*, 1993) and that the original analysis was flawed (Margaritte *et al.*, 1992). Hall *et al.*, (1992) recently localized the BRCA1 gene to an approximately 8 Cm interval (approximately 8 million basepairs) bounded by Mfd15 (D17S250) on the proximal side and the human GIP gene on the distal side. A slightly narrower interval for the BRCA1 locus, based on publicly available data, was agreed upon at the Chromosome 17 workshop in March of 1992 (Fain, 1992). The size of these regions and the uncertainty associated with them has made it exceedingly difficult to design and implement physical mapping and/or cloning strategies for isolating the BRCA1 gene.

Identification of a breast cancer susceptibility locus would permit the early detection of susceptible individuals and greatly increase our ability to understand the initial steps which lead to cancer. As susceptibility loci are often altered during tumor progression, cloning these genes could also be important in the development of better diagnostic and prognostic products, as well as better cancer therapies.

SUMMARY OF THE INVENTION

The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect a human breast cancer predisposing gene (BRCA1), some alleles of which cause susceptibility to cancer, in particular breast and ovarian cancer. More specifically, the present invention relates to germline mutations in the BRCA1 gene and their use in the diagnosis of predisposition to breast and ovarian cancer. The invention further relates to somatic mutations in the BRCA1 gene in human breast cancer and their use in the diagnosis and prognosis of human breast and ovarian cancer. Additionally, the invention relates to somatic mutations in the BRCA1 gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to the therapy of human cancers which have a mutation in the BRCA1 gene, including gene therapy, protein replacement therapy and protein mimetics. Finally, the invention relates to the screening of drugs for cancer therapy.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagram showing the order of loci neighboring BRCA1 as determined by the chromosome 17 workshop. Figure 1 is reproduced from Fain, 1992.

Figure 2 is a schematic map of YACs which define part of Mfd15-Mfd188 region.

Figure 3 is a schematic map of STS's, P1s and BAC's in the BRCA1 region.

Figure 4 is a schematic map of the genomic structure of the BRCA1 gene.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to the field of human genetics. Specifically, the present invention relates to methods and materials used to isolate and detect a human breast cancer

predisposing gene (BRCA1), some alleles of which cause susceptibility to cancer, in particular breast and ovarian cancer. More specifically, the present invention relates to germline mutations in the BRCA1 gene and their use in the diagnosis of predisposition to breast and ovarian cancer. The invention further relates to somatic mutations in the BRCA1 gene in human breast cancer and their use in the diagnosis and prognosis of human breast and ovarian cancer. Additionally, the invention relates to somatic mutations in the BRCA1 gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to the therapy of human cancers which have a mutation in the BRCA1 gene, including gene therapy, protein replacement therapy and protein mimetics. Finally, the invention relates to the screening of drugs for cancer therapy.

The present invention provides an isolated polynucleotide comprising all, or a portion of the BRCA1 locus or of a mutated BRCA1 locus, preferably at least eight bases and not more than about 100 Kb in length. Such polynucleotides may be antisense polynucleotides. The present invention also provides a recombinant construct comprising such an isolated polynucleotide, for example, a recombinant construct suitable for expression in a transformed host cell.

Also provided by the present invention are methods of detecting a polynucleotide comprising a portion of the BRCA1 locus or its expression product in an analyte. Such methods may further comprise the step of amplifying the portion of the BRCA1 locus, and may further include a step of providing a set of polynucleotides which are primers for amplification of said portion of the BRCA1 locus. The method is useful for either diagnosis of the predisposition to cancer or the diagnosis or prognosis of cancer.

The present invention also provides isolated antibodies, preferably monoclonal antibodies, which specifically bind to an isolated polypeptide comprised of at least five amino acid residues encoded by the BRCA1 locus.

The present invention also provides kits for detecting in an analyte a polynucleotide comprising a portion of the BRCA1 locus, the kits comprising a polynucleotide complementary to the portion of the BRCA1 locus packaged in a suitable container, and instructions for its use.

The present invention further provides methods of preparing a polynucleotide comprising polymerizing nucleotides to yield a sequence comprised of at least eight consecutive nucleotides of the BRCA1 locus; and methods of preparing a polypeptide comprising polymerizing amino acids to yield a sequence comprising at least five amino acids encoded within the BRCA1 locus.

In addition, the present invention provides methods of screening drugs for cancer therapy to identify suitable drugs for restoring BRCA1 gene product function.

Finally, the present invention provides the means necessary for production of gene-based therapies directed at cancer cells. These therapeutic agents may take the form of polynucleotides comprising all or a portion of the BRCA1 locus placed in appropriate vectors or delivered to target cells in more direct ways such that the function of the BRCA1 protein is reconstituted. Therapeutic agents may also take the form of polypeptides based on either a portion of, or the entire protein sequence of BRCA1. These may functionally replace the activity of BRCA1 *in vivo*.

It is a discovery of the present invention that the BRCA1 locus which predisposes individuals to breast cancer and ovarian cancer, is a gene encoding a BRCA1 protein, which has been found to have no significant homology with known protein or DNA sequences. This gene is termed BRCA1 herein. It is a discovery of the present invention that mutations in the BRCA1 locus in the germline are indicative of a predisposition to breast cancer and ovarian cancer. Finally, it is a discovery of the present invention that somatic mutations in the BRCA1 locus are also associated with breast cancer, ovarian cancer and other cancers, which represents an indicator of these cancers or of the prognosis of these cancers. The mutational events of the BRCA1 locus can involve deletions, insertions and point mutations within the coding sequence and the non-coding sequence.

Starting from a region on the long arm of human chromosome 17 of the human genome, 17q, which has a size estimated at about 8 million basepairs, a region which contains a genetic locus, BRCA1, which causes susceptibility to cancer, including breast and ovarian cancer, has been identified.

The region containing the BRCA1 locus was identified using a variety of genetic techniques. Genetic mapping techniques initially defined the BRCA1 region in terms of recombination with genetic markers. Based upon studies of large extended families ("kindreds") with multiple cases of breast cancer (and ovarian cancer cases in some kindreds), a chromosomal region has been pinpointed that contains the BRCA1 gene as well as other putative susceptibility alleles in the BRCA1 locus. Two meiotic breakpoints have been discovered on the distal side of the BRCA1 locus which are expressed as recombinants between genetic markers and the disease, and one recombinant on the proximal side of the BRCA1 locus. Thus, a region which contains the BRCA1 locus is physically bounded by these markers.

The use of the genetic markers provided by this invention allowed the identification of clones which cover the region from a human yeast artificial chromosome (YAC) or a human bacterial artificial chromosome (BAC) library. It also allowed for the identification and preparation of more easily manipulated cosmid, P1 and BAC clones from this region and the construction of a contig

from a subset of the clones. These cosmids, P1s, YACs and BACs provide the basis for cloning the BRCA1 locus and provide the basis for developing reagents effective, for example, in the diagnosis and treatment of breast and/or ovarian cancer. The BRCA1 gene and other potential susceptibility genes have been isolated from this region. The isolation was done using software trapping (a
5 computational method for identifying sequences likely to contain coding exon, from contiguous or discontinuous genomic DNA sequences), hybrid selection techniques and direct screening, with whole or partial cDNA inserts from cosmids, P1s and BACs, in the region to screen cDNA libraries. These methods were used to obtain sequences of loci expressed in breast and other tissue. These candidate loci were analyzed to identify sequences which confer cancer susceptibility. We have discovered that
10 there are mutations in the coding sequence of the BRCA1 locus in kindreds which are responsible for the 17q-linked cancer susceptibility known as BRCA1. This gene was not known to be in this region. The present invention not only facilitates the early detection of certain cancers, so vital to patient survival, but also permits the detection of susceptible individuals before they develop cancer.

Population Resources

15 Large, well-documented Utah kindreds are especially important in providing good resources for human genetic studies. Each large kindred independently provides the power to detect whether a BRCA1 susceptibility allele is segregating in that family. Recombinants informative for localization and isolation of the BRCA1 locus could be obtained only from kindreds large enough to confirm the presence of a susceptibility allele. Large sibships are especially important for studying breast cancer,
20 since penetrance of the BRCA1 susceptibility allele is reduced both by age and sex, making informative sibships difficult to find. Furthermore, large sibships are essential for constructing haplotypes of deceased individuals by inference from the haplotypes of their close relatives.

While other populations may also provide beneficial information, such studies generally require much greater effort, and the families are usually much smaller and thus less informative. Utah's age-
25 adjusted breast cancer incidence is 20% lower than the average U.S. rate. The lower incidence in Utah is probably due largely to an early age at first pregnancy, increasing the probability that cases found in Utah kindreds carry a genetic predisposition.

Genetic Mapping

Given a set of informative families, genetic markers are essential for linking a disease to a
30 region of a chromosome. Such markers include restriction fragment length polymorphisms (RFLPs)

(Botstein *et al.*, 1980), markers with a variable number of tandem repeats (VNTRs) (Jeffreys *et al.*, 1985; Nakamura *et al.*, 1987), and an abundant class of DNA polymorphisms based on short tandem repeats (STRs), especially repeats of CpA (Weber and May, 1989; Litt *et al.*, 1989). To generate a genetic map, one selects potential genetic markers and tests them using DNA extracted from members of the kindreds being studied.

Genetic markers useful in searching for a genetic locus associated with a disease can be selected on an *ad hoc* basis, by densely covering a specific chromosome, or by detailed analysis of a specific region of a chromosome. A preferred method for selecting genetic markers linked with a disease involves evaluating the degree of informativeness of kindreds to determine the ideal distance between genetic markers of a given degree of polymorphism, then selecting markers from known genetic maps which are ideally spaced for maximal efficiency. Informativeness of kindreds is measured by the probability that the markers will be heterozygous in unrelated individuals. It is also most efficient to use STR markers which are detected by amplification of the target nucleic acid sequence using PCR; such markers are highly informative, easy to assay (Weber and May, 1989), and can be assayed simultaneously using multiplexing strategies (Skolnick M.H., and Wallace B.R., 1988), greatly reducing the number of experiments required.

Once linkage has been established, one needs to find markers that flank the disease locus, i.e., one or more markers proximal to the disease locus, and one or more markers distal to the disease locus. Where possible, candidate markers can be selected from a known genetic map. Where none is known, new markers can be identified by the STR technique, as shown in the Examples.

Genetic mapping is usually an iterative process. In the present invention, it began by defining flanking genetic markers around the BRCA1 locus, then replacing these flanking markers with other markers that were successively closer to the BRCA1 locus. As an initial step, recombination events, defined by large extended kindreds, helped specifically to localize the BRCA1 locus as either distal or proximal to a specific genetic marker (Goldgar *et al.*, 1993).

The region surrounding BRCA1, until the disclosure of the present invention, was not well mapped and there were few markers. Therefore, short repetitive sequences on cosmids subcloned from YACs, which had been physically mapped, were analyzed in order to develop new genetic markers. Using this approach, one marker of the present invention, 42D6, was discovered which replaced pCMM86 as the distal flanking marker for the BRCA1 region. Since 42D6 is approximately 14 Cm from pCMM86, the BRCA1 region was thus reduced by approximately 14 centimorgans (Easton *et al.*, 1993). The present invention thus began by finding a much more closely linked distal

flanking marker of the BRCA1 region. BRCA1 was then discovered to be distal to the genetic marker Mfd15. Therefore, BRCA1 was shown to be in a region of 6 to 10 million bases bounded by Mfd15 and 42D6. Marker Mfd191 was subsequently discovered to be distal to Mfd15 and proximal to BRCA1. Thus, Mfd15 was replaced with Mfd191 as the closest proximal genetic marker. Similarly, it was discovered that genetic marker Mfd188 could replace genetic marker 42D6, narrowing the region containing the BRCA1 locus to approximately 1.5 million bases. Then the marker Mfd191 was replaced with tdj1474 as the proximal marker and Mfd188 was replaced with U5R as the distal marker, further narrowing the BRCA1 region to a small enough region to allow isolation and characterization of the BRCA1 locus (see Figure 7), using techniques known in the art and described herein.

Physical Mapping

Three distinct methods were employed to physically map the region. The first was the use of yeast artificial chromosomes (YACs) to clone the region which is flanked by tdj1474 and U5R. The second was the creation of a set of P1, BAC and cosmid clones which cover the region containing the BRCA1 locus.

Yeast Artificial Chromosomes (YACs). Once a sufficiently small region containing the BRCA1 locus was identified, physical isolation of the DNA in the region proceeded by identifying a set of overlapping YACs which covers the region. Useful YACs can be isolated from known libraries, such as the St. Louis and CEPH YAC libraries, which are widely distributed and contain approximately 50,000 YACs each. The YACs isolated were from these publicly accessible libraries and can be obtained from a number of sources including the Michigan Genome Center. Clearly, others who had access to these YACS, without the disclosure of the present invention, would not have known the value of the specific YACs we selected since they would not have known which YACs were within, and which YACs outside of, the smallest region containing the BRCA1 locus.

Cosmid, P1 and BAC Clones. In the present invention, it is advantageous to proceed by obtaining cosmid, P1, and BAC clones to cover this region. The smaller size of these inserts, compared to YAC inserts, makes them more useful as specific hybridization probes. Furthermore, having the cloned DNA in bacterial cells, rather than in yeast cells, greatly increases the ease with which the DNA of interest can be manipulated, and improves the signal-to-noise ratio of hybridization assays. For cosmid subclones of YACs, the DNA is partially digested with the restriction enzyme Sau3A and cloned into the BamHI site of the pWE15 cosmid vector (Stratagene,

cat. #1251201). The cosmids containing human sequences are screened by hybridization with human repetitive DNA (e.g., Gibco/BRL, Human Cot-1 DNA, cat. 5279SA), and then fingerprinted by a variety of techniques, as detailed in the Examples.

P1 and BAC clones are obtained by screening libraries constructed from the total human genome with specific sequence tagged sites (STSs) derived from the YACs, cosmids or P1s and BACs, isolated as described herein.

These P1, BAC and cosmid clones can be compared by interspersed repetitive sequence (IRS) PCR and/or restriction enzyme digests followed by gel electrophoresis and comparison of the resulting DNA fragments ("fingerprints"). (Maniatis *et al.*, 1982). The clones can also be characterized by the presence of STSs. The fingerprints are used to define an overlapping contiguous set of clones which covers the region but is not excessively redundant, referred to herein as a "minimum tiling path". Such a minimum tiling path forms the basis for subsequent experiments to identify cDNAs which may originate from the BRCA1 locus.

Coverage of the Gap with P1 and BAC Clones

To cover any gaps in the BRCA1 contig between the identified cosmids with genomic clones, clones in P1 and BAC vectors which contain inserts of genomic DNA roughly twice as large as cosmids for P1s and still greater for BACs (Sternberg, 1990; Sternberg *et al.*, 1990; Pierce *et al.*, 1992; Shizuya *et al.*, (1992)) were used. P1 clones were isolated by Genome Sciences using PCR primers provided by us for screening. BACs were provided by hybridization techniques in Dr. Mel Simon's laboratory. The strategy of using P1 clones also permitted the covering of the genomic region with an independent set of clones not derived from YACs. This guards against the possibility of other deletions in YACs that have not been detected. These new sequences derived from the P1 clones provide the material for further screening for candidate genes, as described below.

Gene Isolation. There are many techniques for testing genomic clones for the presence of sequences likely to be candidates for the coding sequence of a locus one is attempting to isolate, including but not limited to:

- a. zoo blots
- b. identifying HTF islands
- c. exon trapping
- d. hybridizing cDNA to cosmids or YACs.

e. screening cDNA libraries.

(a) Zoo blots. The first technique is to hybridize cosmids to Southern blots to identify DNA sequences which are evolutionarily conserved, and which therefore give positive hybridization signals with DNA from species of varying degrees of relationship to humans (such as monkey, cow, chicken, pig, mouse and rat). Southern blots containing such DNA from a variety of species are commercially available (Clontech, Cat. 7753-1).

(b) Identifying HTF islands. The second technique involves finding regions rich in the nucleotides C and G, which often occur near or within coding sequences. Such sequences are called HTF (HpaI tiny fragment) or CpG islands, as restriction enzymes which contain CpG dimers cut frequently in these regions (Lindsay *et al.*, 1987).

(c) Exon trapping. The third technique is exon trapping, a method that identifies sequences in genomic DNA which contain splice junctions and therefore are likely to comprise coding sequences of genes. Exon amplification (Buckler *et al.*, 1988) is used to select and amplify exons from DNA clones described above. Exon amplification is based on the selection of RNA sequences which are flanked by functional 5' and/or 3' splice sites. The products of the exon amplification are used to screen the breast cDNA libraries to identify a manageable number of candidate genes for further study. Exon trapping can also be performed on small segments of sequenced DNA using computer programs or by software trapping.

(d) Hybridizing cDNA to Cosmids, P1s, BACs or YACS. The fourth technique is a modification of the selective enrichment technique which utilizes hybridization of cDNA to cosmids, P1s, BACs or YACs and permits transcribed sequences to be identified in, and recovered from cloned genomic DNA (Kandpal *et al.*, 1990). The selective enrichment technique, as modified for the present purpose, involves binding DNA from the region of BRCA1 present in a YAC to a column matrix and selecting cDNAs from the relevant libraries which hybridize with the bound DNA, followed by amplification and purification of the bound DNA, resulting in a great enrichment for cDNAs in the region represented by the cloned genomic DNA.

(e) Identification of cDNAs. The fifth technique is to identify cDNAs that correspond to the BRCA1 locus. Hybridization probes containing putative coding sequences, selected using any of the above techniques, are used to screen various libraries, including breast tissue cDNA libraries, ovarian cDNA libraries, and any other necessary libraries.

Another variation on the theme of direct selection of cDNA was also used to find candidate genes for BRCA1 (Lovett *et al.*, 1991; Futreal, 1993). This method uses cosmid, P1 or BAC DNA as the probe. The probe DNA is digested with a blunt cutting restriction enzyme such as HaeIII. Double stranded adaptors are then ligated onto the DNA and serve as binding sites for primers in subsequent PCR amplification reactions using biotinylated primers. Target cDNA is generated from mRNA derived from tissue samples, e.g., breast tissue, by synthesis of either random primed or oligo(dT) primed first strand followed by second strand synthesis. The cDNA ends are rendered blunt and ligated onto double-stranded adaptors. These adaptors serve as amplification sites for PCR. The target and probe sequences are denatured and mixed with human Cot-1 DNA to block repetitive sequences. Solution hybridization is carried out to high Cot-1/2 values to ensure hybridization of rare target cDNA molecules. The annealed material is then captured on avidin beads, washed at high stringency and the retained cDNAs are eluted and amplified by PCR. The selected cDNA is subjected to further rounds of enrichment before cloning into a plasmid vector for analysis.

Testing the cDNA for Candidacy

Proof that the cDNA is the BRCA1 locus is obtained by finding sequences in DNA extracted from affected kindred members which create abnormal BRCA1 gene products or abnormal levels of BRCA1 gene product. Such BRCA1 susceptibility alleles will co-segregate with the disease in large kindreds. They will also be present at a much higher frequency in non-kindred individuals with breast and ovarian cancer than in individuals in the general population. Finally, since tumors often mutate somatically at loci which are in other instances mutated in the germ line, we expect to see normal germ line BRCA1 alleles mutated into sequences which are identical or similar to BRCA1 susceptibility alleles in DNA extracted from tumor tissue. Whether one is comparing BRCA1 sequences from tumor tissue to BRCA1 alleles from the germ line of the same individuals, or one is comparing germ line BRCA1 alleles from cancer cases to those from unaffected individuals, the key is to find mutations which are serious enough to cause obvious disruption to the normal function of the gene product. These mutations can take a number of forms. The most severe forms would be frame shift mutations or large deletions which would cause the gene to code for an abnormal protein or one which would significantly alter protein expression. Less severe disruptive mutations would include small in-frame deletions and nonconservative base pair substitutions which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic to an acidic amino acid or vice versa, from a hydrophobic to hydrophilic amino acid or vice

versa, or other mutations which would affect secondary, tertiary or quaternary protein structure. Silent mutations or those resulting in conservative amino acid substitutions would not generally be expected to disrupt protein function.

According to the diagnostic and prognostic method of the present invention, alteration of the wild-type BRCA1 locus is detected. In addition, the method can be performed by detecting the wild-type BRCA1 locus and confirming the lack of a predisposition to neoplasia. "Alteration of a wild-type gene" encompasses all forms of mutations including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gene or of only a portion of the gene. Point mutations may result in stop codons, frameshift mutations or amino acid substitutions. Somatic mutations are those which occur only in certain tissues, e.g., in the tumor tissue, and are not inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. If only a single allele is somatically mutated, an early neoplastic state is indicated. However, if both alleles are somatically mutated, then a late neoplastic state is indicated. The finding of BRCA1 mutations thus provides both diagnostic and prognostic information. A BRCA1 allele which is not deleted (e.g., found on the sister chromosome to a chromosome carrying a BRCA1 deletion) can be screened for other mutations, such as insertions, small deletions, and point mutations. It is believed that many mutations found in tumor tissues will be those leading to decreased expression of the BRCA1 gene product. However, mutations leading to non-functional gene products would also lead to a cancerous state. Point mutational events may occur in regulatory regions, such as in the promoter of the gene, leading to loss or diminution of expression of the mRNA. Point mutations may also abolish proper RNA processing, leading to loss of expression of the BRCA1 gene product, or to a decrease in mRNA stability or translation efficiency.

Useful diagnostic techniques include, but are not limited to fluorescent *in situ* hybridization (FISH), direct DNA sequencing, PFGE analysis, Southern blot analysis, single stranded conformation analysis (SSCA), RNase protection assay, allele-specific oligonucleotide (ASO), dot blot analysis and PCR-SSCP, as discussed in detail further below.

Predisposition to cancers, such as breast and ovarian cancer, and the other cancers identified herein, can be ascertained by testing any tissue of a human for mutations of the BRCA1 gene. For example, a person who has inherited a germline BRCA1 mutation would be prone to develop cancers. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the BRCA1

gene. Alteration of a wild-type BRCA1 allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

In order to detect the alteration of the wild-type BRCA1 gene in a tissue, it is helpful to isolate the tissue free from surrounding normal tissues. Means for enriching tissue preparation for tumor cells are known in the art. For example, the tissue may be isolated from paraffin or cryostat sections. Cancer cells may also be separated from normal cells by flow cytometry. These techniques, as well as other techniques for separating tumor cells from normal cells, are well known in the art. If the tumor tissue is highly contaminated with normal cells, detection of mutations is more difficult.

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of cancer cases, tumors, or both. Southern blots displaying hybridizing fragments (differing in length from control DNA when probed with sequences near or including the BRCA1 locus) indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the BRCA1 allele(s) and sequencing the allele(s) using techniques well known in the art. Alternatively, the gene sequences can be amplified directly from a genomic DNA preparation from the tumor tissue, using known techniques. The DNA sequence of the amplified sequences can then be determined.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCA) (Orita *et al.*, 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell *et al.*, 1990; Sheffield *et al.*, 1989); 3) RNase protection assays (Finkelstein *et al.*, 1990; Kinszler *et al.*, 1991); 4) allele-specific oligonucleotides (ASOs) (Conner *et al.*, 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and, 6) allele-specific PCR (Rano & Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular BRCA1 mutation. If the particular BRCA1 mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton *et al.*, 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method

is particularly useful for screening relatives of an affected individual for the presence of the BRCA1 mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCA, DGGE and RNase protection assay), a new electrophoretic band appears. SSCA detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type BRCA1 gene coding sequence. The riboprobe and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the BRCA1 mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the BRCA1 mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton *et al.*, 1988; Shenk *et al.*, 1975; Novack *et al.*, 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched

duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the BRCA1 gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

DNA sequences of the BRCA1 gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the BRCA1 gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the BRCA1 gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the BRCA1 gene. Hybridization of allele-specific probes with amplified BRCA1 sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the allele-specific probe.

The most definitive test for mutations in a candidate locus is to directly compare genomic BRCA1 sequences from cancer patients with those from a control population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from cancer patients falling outside the coding region of BRCA1 can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the BRCA1 gene. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in cancer patients as compared to control individuals.

Alteration of BRCA1 mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type BRCA1 gene. Alteration of wild-type BRCA1 genes can also be detected by screening for alteration of wild-type BRCA1 protein. For example, monoclonal antibodies immunoreactive with BRCA1 can be used to screen a tissue. Lack of cognate antigen would indicate a BRCA1 mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant BRCA1 gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and

ELISA assays. Any means for detecting an altered BRCA1 protein can be used to detect alteration of wild-type BRCA1 genes. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect BRCA1 biochemical function. Finding a mutant BRCA1 gene product indicates alteration of a wild-type BRCA1 gene.

5 Mutant BRCA1 genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant BRCA1 genes or gene products in tissues can be applied to other body samples. Cancer cells are sloughed off from tumors and appear in such body samples. In addition, the BRCA1 gene product itself may be secreted into the extracellular space and found in these body samples even in the
10 absence of cancer cells. By screening such body samples, a simple early diagnosis can be achieved for many types of cancers. In addition, the progress of chemotherapy or radiotherapy can be monitored more easily by testing such body samples for mutant BRCA1 genes or gene products.

The methods of diagnosis of the present invention are applicable to any tumor in which BRCA1 has a role in tumorigenesis. The diagnostic method of the present invention is useful for clinicians,
15 so they can decide upon an appropriate course of treatment.

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular BRCA1 allele using PCR. The pairs of single-stranded DNA primers can be annealed to sequences within or surrounding the BRCA1 gene on chromosome 17q21 in order to prime amplifying DNA synthesis of the BRCA1 gene itself. A complete set of these primers allows
20 synthesis of all of the nucleotides of the BRCA1 gene coding sequences, i.e., the exons. The set of primers preferably allows synthesis of both intron and exon sequences. Allele-specific primers can also be used. Such primers anneal only to particular BRCA1 mutant alleles, and thus will only amplify a product in the presence of the mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction
25 enzyme site sequences appended to their 5' ends. Thus, all nucleotides of the primers are derived from BRCA1 sequences or sequences adjacent to BRCA1, except for the few nucleotides necessary to form a restriction enzyme site. Such enzymes and sites are well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Generally, the primers can be made using oligonucleotide synthesizing machines which are commercially available.
30 Given the sequence of the BRCA1 open reading frame shown in SEQ ID NO:1, design of particular primers is well within the skill of the art.

The nucleic acid probes provided by the present invention are useful for a number of purposes. They can be used in Southern hybridization to genomic DNA and in the RNase protection method for detecting point mutations already discussed above. The probes can be used to detect PCR amplification products. They may also be used to detect mismatches with the BRCA1 gene or mRNA using other techniques.

Definitions

The present invention employs the following definitions:

"Amplification of Polynucleotides" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or ligase chain reaction, LCR) and amplification methods based on the use of Q-beta replicase. These methods are well known and widely practiced in the art. See, e.g., U.S. Patents 4,683,195 and 4,683,202 and Innis *et al.*, 1990 (for PCR); and Wu *et al.*, 1989 (for LCR). Reagents and hardware for conducting PCR are commercially available. Primers useful to amplify sequences from the BRCA1 region are preferably complementary to, and hybridize specifically to sequences in the BRCA1 region or in regions that flank a target region therein. BRCA1 sequences generated by amplification may be sequence directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to sequence analysis. A method for the direct cloning and sequence analysis of enzymatically amplified genomic segments has been described by Scharf, 1986.

"Analyte polynucleotide" and **"analyte strand"** refer to a single- or double-stranded polynucleotide which is suspected of containing a target sequence, and which may be present in a variety of types of samples, including biological samples.

"Antibodies." The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and immunologic binding equivalents thereof, which are capable of specifically binding to the BRCA1 polypeptides and fragments thereof or to polynucleotide sequences from the BRCA1 region, particularly from the BRCA1 locus or a portion thereof. The term **"antibody"** is used both to refer to a homogeneous molecular entity, or a mixture such as a serum product made up of a plurality of different molecular entities. Polypeptides may be prepared synthetically in a peptide synthesizer and coupled to a carrier molecule (e.g., keyhole limpet hemocyanin) and injected over several months into rabbits. Rabbit sera is tested for immunoreactivity to the BRCA1 polypeptide or fragment. Monoclonal antibodies may be made by injecting mice with the protein polypeptides, fusion proteins or fragments thereof. Monoclonal antibodies will be screened by ELISA and tested

for specific immunoreactivity with BRCA1 polypeptide or fragments thereof. See, Harlow & Lane, 1988. These antibodies will be useful in assays as well as pharmaceuticals.

Once a sufficient quantity of desired polypeptide has been obtained, it may be used for various purposes. A typical use is the production of antibodies specific for binding. These antibodies may be either polyclonal or monoclonal, and may be produced by *in vitro* or *in vivo* techniques well known in the art. For production of polyclonal antibodies, an appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the immune system in a fashion determined by methods appropriate for the animal and by other parameters well known to immunologists. Typical sites for injection are in footpads, intramuscularly, intraperitoneally, or intradermally. Of course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques known in the art, adjusted for the desired specificity.

An immunological response is usually assayed with an immunoassay. Normally, such immunoassays involve some purification of a source of antigen, for example, that produced by the same cells and in the same fashion as the antigen. A variety of immunoassay methods are well known in the art. See, e.g., Harlow & Lane, 1988, or Goding, 1986.

Monoclonal antibodies with affinities of 10^{-8} M^{-1} or preferably 10^{-9} to 10^{-10} M^{-1} or stronger will typically be made by standard procedures as described, e.g., in Harlow & Lane, 1988 or Goding, 1986. Briefly, appropriate animals will be selected and the desired immunization protocol followed. After the appropriate period of time, the spleens of such animals are excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. Thereafter, the cells are clonally separated and the supernatants of each clone tested for their production of an appropriate antibody specific for the desired region of the antigen.

Other suitable techniques involve *in vitro* exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selection of libraries of antibodies in phage or similar vectors. See Huse *et al.*, 1989. The polypeptides and antibodies of the present invention may be used with or without modification. Frequently, polypeptides and antibodies will be labeled by joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and conjugation techniques are known and are reported extensively in both the scientific and patent literature. Suitable labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent agents, chemiluminescent agents, magnetic particles and the like. Patents teaching the use of such labels include U.S. Patents 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437;

4,275,149; and 4,366,241. Also, recombinant immunoglobulins may be produced (see U.S. Patent 4,816,567).

"Binding partner" refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen and an antigen-specific antibody or an enzyme and its inhibitor. In general, the specific binding partners must bind with sufficient affinity to immobilize the analyte copy/ complementary strand duplex (in the case of polynucleotide hybridization) under the isolation conditions. Specific binding partners are known in the art and include, for example, biotin and avidin or streptavidin, IgG and protein A, the numerous, known receptor-ligand couples, and complementary polynucleotide strands. In the case of complementary polynucleotide binding partners, the partners are normally at least about 15 bases in length, and may be at least 40 bases in length. The polynucleotides may be composed of DNA, RNA, or synthetic nucleotide analogs.

A **"biological sample"** refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide from an individual including, but not limited to, e.g., plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, blood cells, tumors, organs, tissue and samples of *in vitro* cell culture constituents.

As used herein, the terms **"diagnosing"** or **"prognosing,"** as used in the context of neoplasia, are used to indicate 1) the classification of lesions as neoplasia, 2) the determination of the severity of the neoplasia, or 3) the monitoring of the disease progression, prior to, during and after treatment.

"Encode". A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for and/or the polypeptide or a fragment thereof. The anti-sense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

"Isolated" or "substantially pure". An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer) is one which is substantially separated from other cellular components which naturally accompany a native human sequence or protein, e.g., ribosomes, polymerases, many other human genome sequences and proteins. The term embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and includes recombinant or cloned DNA isolates and chemically synthesized analogs or analogs biologically synthesized by heterologous systems.

"BRCA1 Allele" refers to normal alleles of the BRCA1 locus as well as alleles carrying variations that predispose individuals to develop cancer of many sites including, for example, breast,

ovarian, colorectal and prostate cancer. Such predisposing alleles are also called "BRCA1 susceptibility alleles".

"BRCA1 Locus," "BRCA1 gene," "BRCA1 Nucleic Acids" or "BRCA1 Polynucleotide" refer to polynucleotides, all of which are in the BRCA1 region, that are likely to be expressed in normal tissue, certain alleles of which predispose an individual to develop breast, ovarian, colorectal and prostate cancers. Mutations at the BRCA1 locus may be involved in the initiation and/or progression of other types of tumors. The locus is indicated in part by mutations that predispose individuals to develop cancer. These mutations fall within the BRCA1 region described *infra*. The BRCA1 locus is intended to include coding sequences, intervening sequences and regulatory elements controlling transcription and/or translation. The BRCA1 locus is intended to include all allelic variations of the DNA sequence.

These terms, when applied to a nucleic acid, refer to a nucleic acid which encodes a BRCA1 polypeptide, fragment, homolog or variant, including, e.g., protein fusions or deletions. The nucleic acids of the present invention will possess a sequence which is either derived from, or substantially similar to a natural BRCA1-encoding gene or one having substantial homology with a natural BRCA1-encoding gene or a portion thereof. The coding sequence for a BRCA1 polypeptide is shown in SEQ ID NO:1, with the amino acid sequence shown in SEQ ID NO:2.

The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers, both sense and antisense strands, and may be chemically or biochemically modified or may contain non-natural or derivatized nucleotide bases, as will be readily appreciated by those skilled in the art. Such modifications include, for example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as uncharged linkages (e.g., methyl phosphonates, phosphotriesters, phosphoamidates, carbamates, etc.), charged linkages (e.g., phosphorothioates, phosphorodithioates, etc.), pendant moieties (e.g., polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeric nucleic acids, etc.). Also included are synthetic molecules that mimic polynucleotides in their ability to bind to a designated sequence via hydrogen bonding and other chemical interactions. Such molecules are known in the art and include, for example, those in which peptide linkages substitute for phosphate linkages in the backbone of the molecule.

The present invention provides recombinant nucleic acids comprising all or part of the BRCA1 region. The recombinant construct may be capable of replicating autonomously in a host cell.

Alternatively, the recombinant construct may become integrated into the chromosomal DNA of the host cell. Such a recombinant polynucleotide comprises a polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not associated with all or a portion of a polynucleotide with which it is associated in nature; 2) is linked to a polynucleotide other than that to which it is linked in nature; or 3) does not occur in nature.

Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this invention. Although the wild-type sequence may be employed, it will often be altered, e.g., by deletion, substitution or insertion.

cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present invention, or such nucleic acids may be provided by amplification of sequences resident in genomic DNA or other natural sources, e.g., by PCR. The choice of cDNA libraries normally corresponds to a tissue source which is abundant in mRNA for the desired proteins. Phage libraries are normally preferred, but other types of libraries may be used. Clones of a library are spread onto plates, transferred to a substrate for screening, denatured and probed for the presence of desired sequences.

The DNA sequences used in this invention will usually comprise at least about five codons (15 nucleotides), more usually at least about 7-15 codons, and most preferably, at least about 35 codons. One or more introns may also be present. This number of nucleotides is usually about the minimal length required for a successful probe that would hybridize specifically with a BRCA1-encoding sequence.

Techniques for nucleic acid manipulation are described generally, for example, in Sambrook *et al.*, 1989 or Ausebel *et al.*, 1992. Reagents useful in applying such techniques, such as restriction enzymes and the like, are widely known in the art and commercially available from such vendors as New England BioLabs, Boehringer Mannheim, Amersham, Promega Biotec, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid sequences used to produce fusion proteins of the present invention may be derived from natural or synthetic sequences. Many natural gene sequences are obtainable from various cDNA or from genomic libraries using appropriate probes. See, GenBank, National Institutes of Health.

"BRCA1 Region" refers to a portion of human chromosome 17q21 bounded by the markers tdj1474 and U5R. This region contains the BRCA1 locus, including the BRCA1 gene.

As used herein, the terms "**BRCA1 locus**," "**BRCA1 allele**" and "**BRCA1 region**" all refer to the double-stranded DNA comprising the locus, allele, or region, as well as either of the single-stranded DNAs comprising the locus, allele or region.

As used herein, a "**portion**" of the BRCA1 locus or region or allele is defined as having a minimal size of at least about eight nucleotides, or preferably about 15 nucleotides, or more preferably at least about 25 nucleotides, and may have a minimal size of at least about 40 nucleotides.

"**BRCA1 protein**" or "**BRCA1 polypeptide**" refer to a protein or polypeptide encoded by the BRCA1 locus, variants or fragments thereof. The term "polypeptide" refers to a polymer of amino acids and its equivalent and does not refer to a specific length of the product; thus, peptides, oligopeptides and proteins are included within the definition of a polypeptide. This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phosphorylations, and the like. Included within the definition are, for example, polypeptides containing one or more analogs of an amino acid (including, for example, unnatural amino acids, etc.), polypeptides with substituted linkages as well as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at least about 50% homologous to the native BRCA1 sequence, preferably in excess of about 90%, and more preferably at least about 95% homologous. Also included are proteins encoded by DNA which hybridize under high or low stringency conditions, to BRCA1-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera to the BRCA1 protein(s).

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at least about 20 residues, more usually at least about 24 residues, typically at least about 28 residues, and preferably more than about 35 residues.

"**Operably linked**" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. For instance, a promoter is operably linked to a coding sequence if the promoter affects its transcription or expression.

"**Probes**". Polynucleotide polymorphisms associated with BRCA1 alleles which predispose to certain cancers or are associated with most cancers are detected by hybridization with a polynucleotide probe which forms a stable hybrid with that of the target sequence, under stringent to moderately stringent hybridization and wash conditions. If it is expected that the probes will be perfectly complementary to the target sequence, stringent conditions will be used. Hybridization stringency may be lessened if some mismatching is expected, for example, if variants are expected

with the result that the probe will not be completely complementary. Conditions are chosen which rule out nonspecific/adventitious bindings, that is, which minimize noise. Since such indications identify neutral DNA polymorphisms as well as mutations, these indications need further analysis to demonstrate detection of a BRCA1 susceptibility allele.

5 Probes for BRCA1 alleles may be derived from the sequences of the BRCA1 region or its cDNAs. The probes may be of any suitable length, which span all or a portion of the BRCA1 region, and which allow specific hybridization to the BRCA1 region. If the target sequence contains a sequence identical to that of the probe, the probes may be short, e.g., in the range of about 8-30 base pairs, since the hybrid will be relatively stable under even stringent conditions. If some degree
10 of mismatch is expected with the probe, i.e., if it is suspected that the probe will hybridize to a variant region, a longer probe may be employed which hybridizes to the target sequence with the requisite specificity.

The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate other polynucleotide sequences, having sequence similarity by standard
15 methods. For techniques for preparing and labelling probes see, e.g., Sambrook *et al.*, 1989 or Ausebel *et al.*, 1992. Other similar polynucleotides may be selected by using homologous polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthesized or selected by use of the redundancy in the genetic code. Various codon substitutions may be introduced, e.g., by silent changes (thereby producing various restriction sites) or to optimize
20 expression for a particular system. Mutations may be introduced to modify the properties of the polypeptide, perhaps to change ligand-binding affinities, interchain affinities, or the polypeptide degradation or turnover rate.

Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from naturally occurring or recombinant single- or double-stranded polynucleotides,
25 or be chemically synthesized. Probes may also be labelled by nick translation, Klenow fill-in reaction, or other methods known in the art.

Portions of the polynucleotide sequence having at least about eight nucleotides, usually at least about 15 nucleotides, and fewer than about 6 Kb, usually fewer than about 1.0 Kb, from a polynucleotide sequence encoding BRCA1 are preferred as probes. The probes may also be used to
30 determine whether mRNA encoding BRCA1 is present in a cell or tissue.

"Protein modifications or fragments" are provided by the present invention for BRCA1 polypeptides or fragments thereof which are substantially homologous to primary structural sequence but

which include, e.g., *in vivo* or *in vitro* chemical and biochemical modifications or which incorporate unusual amino acids. Such modifications include, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labelling, e.g., with radionuclides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the art, and include radioactive isotopes such as ^{32}P , ligands, which bind to labeled antiligands (e.g., antibodies), fluorophores, chemiluminescent agents, enzymes, and antiligands which can serve as specific binding pair members for a labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability requirements, and available instrumentation. Methods of labelling polypeptides are well known in the art. See, e.g., Sambrook *et al.*, 1989 or Ausebel *et al.*, 1992.

Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polypeptides. Significant biological activities include ligand-binding, immunological activity and other biological activities characteristic of BRCA1 polypeptides. Immunological activities include both immunogenic function in a target immune system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an epitope of the BRCA1 protein. As used herein, "epitope" refers to an antigenic determinant of a polypeptide. An epitope could comprise three amino acids in a spatial conformation which is unique to the epitope. Generally, an epitope consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determining the spatial conformation of such amino acids are known in the art.

For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highly antigenic proteins. Alternatively, such polypeptides will serve as highly efficient competitors for specific binding. Production of antibodies specific for BRCA1 polypeptides or fragments thereof is described below.

The present invention also provides for fusion polypeptides, comprising BRCA1 polypeptides and fragments. Homologous polypeptides may be fusions between two or more BRCA1 polypeptide sequences or between the sequences of BRCA1 and a related protein. Likewise, heterologous fusions may be constructed which would exhibit a combination of properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between different new fusion polypeptides or fragments. Such homologous or heterologous fusion polypeptides may display, for example, altered strength or specificity of binding. Fusion partners include

immunoglobulins, bacterial β -galactosidase, trpE, protein A, β -lactamase, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor. See, e.g., Godowski *et al.*, 1988.

5 Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemically synthesized. Techniques for the synthesis of polypeptides are described, for example, in Merrifield, 1963.

10 **"Protein purification"** refers to various methods for the isolation of the BRCA1 polypeptides from other biological material, such as from cells transformed with recombinant nucleic acids encoding BRCA1, and are well known in the art. For example, such polypeptides may be purified by immunoaffinity chromatography employing, e.g., the antibodies provided by the present invention. Various methods of protein purification are well known in the art, and include those described in Deutscher, 1990 and Scopes, 1982.

15 The terms **"isolated"**, **"substantially pure"**, and **"substantially homogenous"** are used interchangeably to describe a protein or polypeptide which has been separated from components which accompany it in its natural state. A monomeric protein is substantially pure when at least about 60 to 75% of a sample exhibits a single polypeptide sequence. A substantially pure protein will typically comprise about 60 to 90% W/W of a protein sample, more usually about 95%, and preferably will be over about 99% pure. Protein purity or homogeneity may be indicated by a number of means well known in the art, such as polyacrylamide gel electrophoresis or a protein sample, followed by visualizing a single polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other means well known in the art for purification utilized.

20 A BRCA1 protein is substantially free of naturally associated components when it is separated from the native contaminants which accompany it in its natural state. Thus, a polypeptide which is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be substantially free from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

30 A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypeptide," as used herein, even if expressed in a homologous cell type. Synthetically made forms or molecules expressed by heterologous cells are inherently isolated molecules.

"Recombinant nucleic acid" is a nucleic acid which is not naturally occurring, or which is made by the artificial combination of two otherwise separated segments of sequence. This artificial combination is often accomplished by either chemical synthesis means, or by the artificial manipulation of isolated segments of nucleic acids, e.g., by genetic engineering techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino acid, while typically introducing or removing a sequence recognition site. Alternatively, it is performed to join together nucleic acid segments of desired functions to generate a desired combination of functions.

"Regulatory sequences" refers to those sequences normally within 100 Kb of the coding region of a locus, but they may also be more distant from the coding region, which affect the expression of the gene (including transcription of the gene, and translation, splicing, stability or the like of the messenger RNA).

"Substantial homology or similarity". A nucleic acid or fragment thereof is "substantially homologous" ("or substantially similar") to another if, when optimally aligned (with appropriate nucleotide insertions or deletions) with the other nucleic acid (or its complementary strand), there is nucleotide sequence identity in at least about 60% of the nucleotide bases, usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least about 95-98% of the nucleotide bases.

Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nucleic acid (or a complementary strand thereof) under selective hybridization conditions, to a strand, or to its complement. Selectivity of hybridization exists when hybridization which is substantially more selective than total lack of specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of at least about 14 nucleotides, preferably at least about 65%, more preferably at least about 75%, and most preferably at least about 90%. See, Kanehisa, 1984. The length of homology comparison, as described, may be over longer stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 20 nucleotides, more usually at least about 24 nucleotides, typically at least about 28 nucleotides, more typically at least about 32 nucleotides, and preferably at least about 36 or more nucleotides.

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in addition to the base composition, length of the complementary strands, and the number of nucleotide base mismatches between the hybridizing nucleic acids, as will

be readily appreciated by those skilled in the art. Stringent temperature conditions will generally include temperatures in excess of 30°C, typically in excess of 37°C, and preferably in excess of 45°C. Stringent salt conditions will ordinarily be less than 1000 mM, typically less than 500 mM, and preferably less than 200 mM. However, the combination of parameters is much more important than the measure of any single parameter. See, e.g., Wetmur & Davidson, 1968.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher order DNA complexes. The preparation of such probes and suitable hybridization conditions are well known in the art.

The terms "**substantial homology**" or "**substantial identity**", when referring to polypeptides, indicate that the polypeptide or protein in question exhibits at least about 30% identity with an entire naturally-occurring protein or a portion thereof, usually at least about 70% identity, and preferably at least about 95% identity.

"**Substantially similar function**" refers to the function of a modified nucleic acid or a modified protein, with reference to the wild-type BRCA1 nucleic acid or wild-type BRCA1 polypeptide. The modified polypeptide will be substantially homologous to the wild-type BRCA1 polypeptide and will have substantially the same function. The modified polypeptide may have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the modified polypeptide may have other useful properties, such as a longer half-life. The similarity of function (activity) of the modified polypeptide may be substantially the same as the activity of the wild-type BRCA1 polypeptide. Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type BRCA1 polypeptide. The modified polypeptide is synthesized using conventional techniques, or is encoded by a modified nucleic acid and produced using conventional techniques. The modified nucleic acid is prepared by conventional techniques. A nucleic acid with a function substantially similar to the wild-type BRCA1 gene function produces the modified protein described above.

Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 910 University Avenue, Madison, Wisconsin 53705. Protein analysis software matches similar sequences using measure of homology assigned to various substitutions, deletions, substitutions, and other modifications. Conservative substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine.

A polypeptide "**fragment**," "**portion**" or "**segment**" is a stretch of amino acid residues of at least about five to seven contiguous amino acids, often at least about seven to nine contiguous amino acids, typically at least about nine to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more contiguous amino acids.

5 The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, column packing materials (e.g., Sepharose beads), magnetic beads, glass wool, plastic, metal, polymer gels, cells, or other substrates. Such supports may take the form, for example, of beads, wells, dipsticks, membranes.

10 "**Target region**" refers to a region of the nucleic acid which is amplified and/or detected. The term "**target sequence**" refers to a sequence with which a probe or primer will form a stable hybrid under desired conditions.

15 The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecular biology, microbiology, recombinant DNA, genetics, and immunology. See, e.g., Maniatis *et al.*, 1982; Sambrook *et al.*, 1989; Ausebel *et al.*, 1992; Glover, 1985; Anand, 1992; Guthrie & Fink, 1991. A general discussion of techniques and materials for human gene mapping, including mapping of human chromosome 17q, is provided, e.g., in White & Lalouel, 1988.

Preparation of recombinant or chemically synthesized
nucleic acids; vectors, transformation, host cells

20 Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also
25 be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook *et al.*, 1989 or Ausebel *et al.*, 1992.

30 The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage & Carruthers, 1981 or the triester method according to Matteucci *et al.*, 1981, and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of

chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may
5 comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and necessary processing
10 information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Secretion signals may also be included where appropriate, whether from a native BRCA1 protein or from other receptors or from secreted polypeptides of the same or related species, which allow the protein to cross and/or lodge in cell membranes, and thus attain its functional topology, or be secreted from the cell. Such
15 vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook *et al.*, 1989 or Ausebel *et al.* 1992.

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with BRCA1 genes. Examples of workable combinations of cell lines and expression vectors are described in
20 Sambrook *et al.*, 1989 or Ausebel *et al.*, 1992; see also, e.g., Metzger *et al.*, 1988. Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes
25 such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others. Vectors and promoters suitable for use in yeast expression are further described in Hitzeman *et al.*, EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers *et al.*, 1978) or promoters derived from murine molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine
30 papilloma virus or polyoma. In addition, the construct may be joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other

expression control sequences, see also *Enhancers and Eukaryotic Gene Expression*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983).

While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

5 Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances, e.g. ampicillin, neomycin, methotrexate, etc.; b) complement auxotrophic deficiencies, or c) supply critical nutrients
10 not available from complex media, e.g., the gene encoding D-alanine racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

 The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo *et al.*, 1988),
15 or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook *et al.*, 1989 and Ausubel *et al.*, 1992. The
20 introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

 Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the BRCA1 nucleic acids or portions thereof in vectors or other expression vehicles
25 in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

 Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present
30 invention. Propagation of mammalian cells in culture is *per se* well known. See, Kruse & Patterson, 1973. Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by

the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of BRCA1 polypeptides.

Antisense polynucleotide sequences are useful in preventing or diminishing the expression of the BRCA1 locus, as will be appreciated by those skilled in the art. For example, polynucleotide vectors containing all or a portion of the BRCA1 locus or other sequences from the BRCA1 region (particularly those flanking the BRCA1 locus) may be placed under the control of a promoter in an antisense orientation and introduced into a cell. Expression of such an antisense construct within a cell will interfere with BRCA1 transcription and/or translation and/or replication.

The probes and primers based on the BRCA1 gene sequences disclosed herein are used to identify homologous BRCA1 gene sequences and proteins in other species. These BRCA1 gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of a BRCA1 allele predisposing an individual to cancer, a biological sample such as blood is prepared and analyzed for the presence or absence of susceptibility alleles of BRCA1. In order to detect the presence of neoplasia, the progression toward malignancy of a precursor lesion, or as a prognostic indicator, a biological sample of the lesion is prepared and analyzed for the presence or absence of mutant alleles of BRCA1. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic laboratories, or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

Initially, the screening method involves amplification of the relevant BRCA1 sequences, e.g., by PCR, followed by DNA sequence analysis.

When the probes are used to detect the presence of the target sequences (for example, in screening for cancer susceptibility), the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of human chromosome 17q. Therefore, high stringency conditions are desirable

in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in, for example, Maniatis *et al.*, 1982 and Sambrook *et al.*, 1989. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadruplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme are known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are reviewed in, e.g., Matthews & Kricka, 1988; Landegren *et al.*, 1988; Mittlin, 1989, U.S. Patent 4,868,105, and in EPO Publication No. 225,807.

Methods of Use: Peptide Diagnosis and Diagnostic Kits

The neoplastic condition of lesions can also be detected on the basis of the alteration of wild-type BRCA1 polypeptide. Such alterations can be determined by sequence analysis in accordance with conventional techniques. More preferably, antibodies (polyclonal or monoclonal) are used to
5 detect differences in, or the absence of BRCA1 peptides. In a preferred embodiment of the invention, antibodies will immunoprecipitate BRCA1 proteins from solution as well as react with BRCA1 protein on Western or immunoblots of polyacrylamide gels. In another preferred embodiment, antibodies will detect BRCA1 proteins in paraffin or frozen tissue sections, using immunocytochemical techniques. Techniques for raising and purifying antibodies are well known
10 in the art, and any such techniques may be chosen to achieve the preparation of the invention.

Methods of Use: Drug Screening

This invention is particularly useful for screening compounds by using the BRCA1 polypeptide or binding fragment thereof in any of a variety of drug screening techniques.

The BRCA1 polypeptide or fragment employed in such a test may either be free in solution,
15 affixed to a solid support, or borne on a cell surface. One method of drug screening utilizes eucaryotic or procaryotic host cells which are stably transformed with recombinant polynucleotides expressing the polypeptide or fragment, preferably in competitive binding assays. Such cells, either in viable or fixed form, can be used for standard binding assays. one may measure, for example, for the formation of complexes between a BRCA1 polypeptide or fragment and the agent being tested,
20 or examine the degree to which the formation of a complex between a BRCA1 polypeptide or fragment and a known ligand is interfered with by the agent being tested.

Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a BRCA1 polypeptide or fragment thereof and assaying (i) for the presence of a complex between the agent and the BRCA1 polypeptide or fragment, or (ii) for the presence of a
25 complex between the BRCA1 polypeptide or fragment and a ligand, by methods well known in the art. In such competitive binding assays the BRCA1 polypeptide or fragment is typically labeled. Free BRCA1 polypeptide or fragment is separated from that present in a protein:protein complex, and the amount of free (i.e., uncomplexed) label is a measure of the binding of the agent being tested to BRCA1 or its interference with BRCA1:ligand binding, respectively.

30 Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to the BRCA1 polypeptides and is described in detail in Geysen, European

Patent Application 84/03564, published on September 13, 1984. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with BRCA1 polypeptide and washed. Bound BRCA1 polypeptide is then detected by methods well known in the art.

5 Purified BRCA1 can be coated directly onto plates for use in the aforementioned drug screening techniques. However, non-neutralizing antibodies to the polypeptide can be used to capture antibodies to immobilize the BRCA1 polypeptide on the solid phase.

10 This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of specifically binding the BRCA1 polypeptide compete with a test compound for binding to the BRCA1 polypeptide or fragments thereof. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants of the BRCA1 polypeptide.

15 A further technique for drug screening involves the use of host eukaryotic cell lines or cells (such as described above) which have a nonfunctional BRCA1 gene. These host cell lines or cells are defective at the BRCA1 polypeptide level. The host cell lines or cells are grown in the presence of drug compound. The rate of growth of the host cells is measured to determine if the compound is capable of regulating the growth of BRCA1 defective cells.

Methods of Use: Rational Drug Design

20 The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small molecules with which they interact (e.g., agonists, antagonists, inhibitors) in order to fashion drugs which are, for example, more active or stable forms of the polypeptide, or which, e.g., enhance or interfere with the function of a polypeptide *in vivo*. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a protein of interest (e.g., BRCA1 polypeptide) or, for example, of the BRCA1-receptor or ligand complex, 25 by x-ray crystallography, by computer modelling or most typically, by a combination of approaches. Less often, useful information regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. An example of rational drug design is the development of HIV protease inhibitors (Erickson *et al.*, 1990). In addition, peptides (e.g., BRCA1 polypeptide) are analyzed by an alanine scan (Wells, 1991). In this technique, an amino acid residue 30 is replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is analyzed in this manner to determine the important regions of the peptide.

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure. In principle, this approach yields a pharmacore upon which subsequent drug design can be based. It is possible to bypass protein crystallography altogether by generating anti-idiotypic antibodies (anti-ids) to a functional, pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected to be an analog of the original receptor. The anti-id could then be used to identify and isolate peptides from banks of chemically or biologically produced banks of peptides. Selected peptides would then act as the pharmacore.

Thus, one may design drugs which have, e.g., improved BRCA1 polypeptide activity or stability or which act as inhibitors, agonists, antagonists, etc. of BRCA1 polypeptide activity. By virtue of the availability of cloned BRCA1 sequences, sufficient amounts of the BRCA1 polypeptide may be made available to perform such analytical studies as X-ray crystallography. In addition, the knowledge of the BRCA1 protein sequence provided herein will guide those employing computer modeling techniques in place of, or in addition to x-ray crystallography.

Methods of Use: Gene Therapy

According to the present invention, a method is also provided of supplying wild-type BRCA1 function to a cell which carries mutant BRCA1 alleles. Supplying such a function should suppress neoplastic growth of the recipient cells. The wild-type BRCA1 gene or a part of the gene may be introduced into the cell in a vector such that the gene remains extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. If a gene fragment is introduced and expressed in a cell carrying a mutant BRCA1 allele, the gene fragment should encode a part of the BRCA1 protein which is required for non-neoplastic growth of the cell. More preferred is the situation where the wild-type BRCA1 gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endogenous mutant BRCA1 gene present in the cell. Such recombination requires a double recombination event which results in the correction of the BRCA1 gene mutation. Vectors for introduction of genes both for recombination and for extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA into cells such as electroporation, calcium phosphate co-precipitation and viral transduction are known in the art, and the choice of method is within the competence of the routineer. Cells transformed with the wild-type BRCA1 gene can be used as model systems to study cancer remission and drug treatments which promote such remission.

As generally discussed above, the BRCA1 gene or fragment, where applicable, may be employed in gene therapy methods in order to increase the amount of the expression products of such genes in cancer cells. Such gene therapy is particularly appropriate for use in both cancerous and pre-cancerous cells, in which the level of BRCA1 polypeptide is absent or diminished compared to normal cells. It may also be useful to increase the level of expression of a given BRCA1 gene even in those tumor cells in which the mutant gene is expressed at a "normal" level, but the gene product is not fully functional.

Gene therapy would be carried out according to generally accepted methods, for example, as described by Friedman, 1991. Cells from a patient's tumor would be first analyzed by the diagnostic methods described above, to ascertain the production of BRCA1 polypeptide in the tumor cells. A virus or plasmid vector (see further details below), containing a copy of the BRCA1 gene linked to expression control elements and capable of replicating inside the tumor cells, is prepared. Suitable vectors are known, such as disclosed in U.S. Patent 5,252,479 and PCT published application WO 93/07282. The vector is then injected into the patient, either locally at the site of the tumor or systemically (in order to reach any tumor cells that may have metastasized to other sites). If the transfected gene is not permanently incorporated into the genome of each of the targeted tumor cells, the treatment may have to be repeated periodically.

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention. These include viral and nonviral transfer methods. A number of viruses have been used as gene transfer vectors, including papovaviruses (e.g., SV40, Madzak *et al.*, 1992), adenovirus (Berkner, 1992; Berkner *et al.*, 1988; Gorziglia and Kapikian, 1992; Quantin *et al.*, 1992; Rosenfeld *et al.*, 1992; Wilkinson *et al.*, 1992; Stratford-Perricaudet *et al.*, 1990), vaccinia virus (Moss, 1992), adeno-associated virus (Muzyczka, 1992; Ohi *et al.*, 1990), herpesviruses including HSV and EBV (Margolskee, 1992; Johnson *et al.*, 1992; Fink *et al.*, 1992; Breakfield and Geller, 1987; Freese *et al.*, 1990), and retroviruses of avian (Brandyopadhyay and Temin, 1984; Petropoulos *et al.*, 1992), murine (Miller, 1992; Miller *et al.*, 1985; Sorge *et al.*, 1984; Mann and Baltimore, 1985; Miller *et al.*, 1988), and human origin (Shimada *et al.*, 1991; Helseth *et al.*, 1990; Page *et al.*, 1990; Buchschacher and Panganiban, 1992). Most human gene therapy protocols have been based on disabled murine retroviruses.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (Graham and van der Eb, 1973; Pellicer *et al.*, 1980); mechanical techniques, for example microinjection (Anderson *et al.*, 1980; Gordon *et al.*, 1980; Brinster *et al.*,

1981; Constantini and Lacy, 1981); membrane fusion-mediated transfer via liposomes (Felgner *et al.*, 1987; Wang and Huang, 1989; Kaneda *et al.*, 1989; Stewart *et al.*, 1992; Nabel *et al.*, 1990; Lim *et al.*, 1992); and direct DNA uptake and receptor-mediated DNA transfer (Wolff *et al.*, 1990; Wu *et al.*, 1991; Zenke *et al.*, 1990; Wu *et al.*, 1989; Wolff *et al.*, 1991; Wagner *et al.*, 1990; Wagner *et al.*, 1991; Cotten *et al.*, 1990; Curiel *et al.*, 1991; Curiel *et al.*, 1991). Viral-mediated gene transfer can be combined with direct in vivo gene transfer using liposome delivery, allowing one to direct the viral vectors to the tumor cells and not into the surrounding nondividing cells. Alternatively, the retroviral vector producer cell line can be injected into tumors (Culver *et al.*, 1992). Injection of producer cells would then provide a continuous source of vector particles. This technique has been approved for use in humans with inoperable brain tumors.

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with a polylysine-conjugated antibody specific to the adenovirus hexon protein, and the resulting complex is bound to an adenovirus vector. The trimolecular complex is then used to infect cells. The adenovirus vector permits efficient binding, internalization, and degradation of the endosome before the coupled DNA is damaged.

Liposome/DNA complexes have been shown to be capable of mediating direct in vivo gene transfer. While in standard liposome preparations the gene transfer process is nonspecific, localized in vivo uptake and expression have been reported in tumor deposits, for example, following direct *in situ* administration (Nabel, 1992).

Gene transfer techniques which target DNA directly to breast and ovarian tissues, e.g., epithelial cells of the breast or ovaries, is preferred. Receptor-mediated gene transfer, for example, is accomplished by the conjugation of DNA (usually in the form of covalently closed supercoiled plasmid) to a protein ligand via polylysine. Ligands are chosen on the basis of the presence of the corresponding ligand receptors on the cell surface of the target cell/tissue type. One appropriate receptor/ligand pair may include the estrogen receptor and its ligand, estrogen (and estrogen analogues). These ligand-DNA conjugates can be injected directly into the blood if desired and are directed to the target tissue where receptor binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DNA, coinfection with adenovirus can be included to disrupt endosome function.

The therapy involves two steps which can be performed singly or jointly. In the first step, prepubescent females who carry a BRCA1 susceptibility allele are treated with a gene delivery vehicle such that some or all of their mammary ductal epithelial precursor cells receive at least one

additional copy of a functional normal BRCA1 allele. In this step, the treated individuals have reduced risk of breast cancer to the extent that the effect of the susceptible allele has been countered by the presence of the normal allele. In the second step of a preventive therapy, predisposed young females, in particular women who have received the proposed gene therapeutic treatment, undergo hormonal therapy to mimic the effects on the breast of a full term pregnancy.

Methods of Use: Peptide Therapy

Peptides which have BRCA1 activity can be supplied to cells which carry mutant or missing BRCA1 alleles. The sequence of the BRCA1 protein is disclosed (SEQ ID NO:2). Protein can be produced by expression of the cDNA sequence in bacteria, for example, using known expression vectors. Alternatively, BRCA1 polypeptide can be extracted from BRCA1-producing mammalian cells. In addition, the techniques of synthetic chemistry can be employed to synthesize BRCA1 protein. Any of such techniques can provide the preparation of the present invention which comprises the BRCA1 protein. The preparation is substantially free of other human proteins. This is most readily accomplished by synthesis in a microorganism or *in vitro*.

Active BRCA1 molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, some active molecules may be taken up by cells, actively or by diffusion. Extracellular application of the BRCA1 gene product may be sufficient to affect tumor growth. Supply of molecules with BRCA1 activity should lead to partial reversal of the neoplastic state. Other molecules with BRCA1 activity (for example, peptides, drugs or organic compounds) may also be used to effect such a reversal. Modified polypeptides having substantially similar function are also used for peptide therapy.

Methods of Use: Transformed Hosts

Similarly, cells and animals which carry a mutant BRCA1 allele can be used as model systems to study and test for substances which have potential as therapeutic agents. The cells are typically cultured epithelial cells. These may be isolated from individuals with BRCA1 mutations, either somatic or germline. Alternatively, the cell line can be engineered to carry the mutation in the BRCA1 allele, as described above. After a test substance is applied to the cells, the neoplastically transformed phenotype of the cell is determined. Any trait of neoplastically transformed cells can be assessed, including anchorage-independent growth, tumorigenicity in nude mice, invasiveness of cells, and growth factor dependence. Assays for each of these traits are known in the art.

Animals for testing therapeutic agents can be selected after mutagenesis of whole animals or after treatment of germline cells or zygotes. Such treatments include insertion of mutant BRCA1 alleles, usually from a second animal species, as well as insertion of disrupted homologous genes. Alternatively, the endogenous BRCA1 gene(s) of the animals may be disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques. (Capecchi, 1989; Valancius and Smithies, 1991; Hasty *et al.*, 1991; Shinkai *et al.*, 1992; Mombaerts *et al.*, 1992; Philpott *et al.*, 1992; Snouwaert *et al.*, 1992; Donehower *et al.*, 1992) After test substances have been administered to the animals, the growth of tumors must be assessed. If the test substance prevents or suppresses the growth of tumors, then the test substance is a candidate therapeutic agent for the treatment of the cancers identified herein. These animal models provide an extremely important testing vehicle for potential therapeutic products.

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described below were utilized.

EXAMPLE 1

Ascertain and Study Kindreds Likely to Have a 17q-Linked Breast Cancer Susceptibility Locus

Extensive cancer prone kindreds were ascertained from a defined population providing a large set of extended kindreds with multiple cases of breast cancer and many relatives available to study. The large number of meioses present in these large kindreds provided the power to detect whether the BRCA1 locus was segregating, and increased the opportunity for informative recombinants to occur within the small region being investigated. This vastly improved the chances of establishing linkage to the BRCA1 region, and greatly facilitated the reduction of the BRCA1 region to a manageable size, which permits identification of the BRCA1 locus itself.

Each kindred was extended through all available connecting relatives, and to all informative first degree relatives of each proband or cancer case. For these kindreds, additional breast cancer cases and individuals with cancer at other sites of interest (e.g. ovarian) who also appeared in the kindreds were identified through the tumor registry linked files. All breast cancers reported in the kindred which were not confirmed in the Utah Cancer Registry were researched. Medical records

or death certificates were obtained for confirmation of all cancers. Each key connecting individual and all informative individuals were invited to participate by providing a blood sample from which DNA was extracted. We also sampled spouses and relatives of deceased cases so that the genotype of the deceased cases could be inferred from the genotypes of their relatives.

5 Ten kindreds which had three or more cancer cases with inferable genotypes were selected for linkage studies to 17q markers from a set of 29 kindreds originally ascertained from the linked databases for a study of proliferative breast disease and breast cancer (Skolnick *et al.*, 1990). The criterion for selection of these kindreds was the presence of two sisters or a mother and her daughter with breast cancer. Additionally, two kindreds which have been studied since 1980 as part of our
10 breast cancer linkage studies (K1001, K9018), six kindreds ascertained from the linked databases for the presence of clusters of breast and/or ovarian cancer (K2019, K2073, K2079, K2080, K2039; K2082; and a self-referred kindred with early onset breast cancer (K2035) were included. These kindreds were investigated and expanded in our clinic in the manner described above. Table 1 displays the characteristics of these 19 kindreds which are the subject of subsequent examples. In
15 Table 1, for each kindred the total number of individuals in our database, the number of typed individuals, and the minimum, median, and maximum age at diagnosis of breast/ovarian cancer are reported. Kindreds are sorted in ascending order of median age at diagnosis of breast cancer. Four women diagnosed with both ovarian and breast cancer are counted in both categories.

TABLE 1

Description of the 19 Kindreds

KINDRED	No. of Individuals		Breast				Ovarian			
			# Aff.	Age at Dx			# Aff.	Age at Dx		
	Total	Sample		Min.	Med.	Max.		Min.	Med.	Max.
1910	15	10	4	27	34	49	-	-	-	-
1001	133	98	13	28	37	64	-	-	-	-
2035	42	25	8	28	37	45	1	-	60	-
2027	21	11	4	34	38	41	-	-	-	-
9018	54	17	9	30	40	72	2	46	48	50
1925	50	27	4	39	42	53	-	-	-	-
1927	49	29	5	32	42	51	-	-	-	-
1911	28	21	7	28	42	76	-	-	-	-
1929	16	11	4	34	43	73	-	-	-	-
1901	35	19	10	31	44	76	-	-	-	-
2082	180	105	20	27	47	67	10	45	52	66
2019	42	19	10	42	53	79	-	-	-	-
1900	70	23	8	45	55	70	1	-	78	-
2080	264	74	22+	27	55	92	4	45	53	71
2073	57	29	9	35	57	80	-	-	-	-
1917	16	6	4	43	58	61	-	-	-	-
1920	22	14	3	62	63	68	-	-	-	-
2079	136	18	14	38	66	84	4	52	59	65
2039	87	40	14	44	68	88	4	41	51	75

44-

+ Includes one case of male breast cancer.

EXAMPLE 2

Selection of Kindreds Which are Linked to Chromosome 17q and Localization of BRCA1 to the Interval Mfd15 - Mfd188

For each sample collected in these 19 kindreds, DNA was extracted from blood (or in two cases
5 from paraffin-embedded tissue blocks) using standard laboratory protocols. Genotyping in this study
was restricted to short tandem repeat (STR) markers since, in general, they have high heterozygosity
and PCR methods offer rapid turnaround while using very small amounts of DNA. To aid in this
effort, four such STR markers on chromosome 17 were developed by screening a chromosome
10 specific cosmid library for CA positive clones. Three of these markers localized to the long arm:
(46E6, Easton *et al.*, 1993); (42D6, Easton *et al.*, 1993); 26C2 (D17S514, Oliphant *et al.*, 1993),
while the other, 12G6, (D17S513, Oliphant *et al.*, 1991) localized to the short arm near the p53
tumor suppressor locus. Two of these, 42D6 and 46E6, were submitted to the Breast Cancer Linkage
Consortium for typing of breast cancer families by investigators worldwide. Oligonucleotide
15 sequences for markers not developed in our laboratory were obtained from published reports, or as
part of the Breast Cancer Linkage Consortium, or from other investigators. All genotyping films
were scored blindly with a standard lane marker used to maintain consistent coding of alleles. Key
samples in the four kindreds presented here underwent duplicate typing for all relevant markers. All
19 kindreds have been typed for two polymorphic CA repeat markers: 42D6 (D17S588), a CA repeat
isolated in our laboratory, and Mfd15 (D17S250), a CA repeat provided by J. Weber (Weber *et al.*,
20 1990). Several sources of probes were used to create genetic markers on chromosome 17,
specifically chromosome 17 cosmid and lambda phage libraries created from sorted chromosomes
by the Los Alamos National Laboratories (van Dilla *et al.*, 1986).

LOD scores for each kindred with these two markers (42D6, Mfd15) and a third marker,
Mfd188 (D17S579, Hall *et al.* 1992), located roughly midway between these two markers, were
25 calculated for two values of the recombination fraction, 0.001 and 0.1. (For calculation of LOD
scores, see OH, 1985). Likelihoods were computed under the model derived by Claus *et al.*, 1991,
which assumes an estimated gene frequency of 0.003, a lifetime risk in gene carriers of about 0.80,
and population based age-specific risks for breast cancer in non-gene carriers. Allele frequencies for
the three markers used for the LOD score calculations were calculated from our own laboratory
30 typings of unrelated individuals in the CEPH panel (White *et al.*, 1988). Table 2 shows the results
of the pairwise linkage analysis of each kindred with the three markers 42D6, Mfd188, and Mfd15.

TABLE 2

Pairwise Linkage Analysis of Kindreds

<u>KINDRED</u>	<u>Mfd15 (D17S250)</u> <u>Recombination</u>		<u>42D6 (D17S588)</u> <u>Recombination</u>		<u>Mfd188 (D17S579)</u> <u>Recombination</u>	
	0.001	0.1	0.001	0.1	0.001	0.1
1910	0.06	0.30	0.06	0.30	0.06	0.30
1001	-0.30	-0.09	NT	NT	-0.52	-0.19
2035	2.34	1.85	0.94	0.90	2.34	1.82
2027	-1.22	-0.33	-1.20	-0.42	-1.16	-0.33
9018	-0.54	-0.22	-0.17	-0.10	0.11	0.07
1925	1.08	0.79	0.55	0.38	-0.11	-0.07
1927	-0.41	0.01	-0.35	0.07	-0.44	-0.02
1911	-0.27	-0.13	-0.43	-0.23	0.49	0.38
1929	-0.49	-0.25	NT	NT	-0.49	-0.25
1901	1.50	1.17	0.78	0.57	0.65	0.37
2082	4.25	3.36	6.07	5.11	2.00	3.56
2019	-0.10	-0.01	-0.11	-0.05	-0.18	-0.10
1900	-0.14	-0.11	NT	NT	-0.12	-0.05
2080	-0.16	-0.04	0.76	0.74	-1.25	-0.58
2073	-0.41	-0.29	0.63	0.49	-0.23	-0.13
1917	-0.02	-0.02	NT	NT	-0.01	0.00
1920	-0.03	-0.02	NT	NT	0.00	0.00
2079	0.02	0.01	-0.01	-0.01	0.01	0.01
2039	-1.67	-0.83	0.12	0.59	-1.15	0.02

NT - Kindred not typed for Mfd188.

Using a criterion for linkage to 17q of a LOD score > 1.0 for at least one locus under the CASH model (Claus *et al.*, 1991), four of the 19 kindreds appeared to be linked to 17q (K1901, K1925, K2035, K2082). A number of additional kindreds showed some evidence of linkage but at this time could not be definitively assigned to the linked category. These included kindreds K1911, K2073, K2039, and K2080. Three of the 17q-linked kindreds had informative recombinants in this region and these are detailed below.

Kindred 2082 is the largest 17q-linked breast cancer family reported to date by any group. The kindred contains 20 cases of breast cancer, and ten cases of ovarian cancer. Two cases have both ovarian and breast cancer. The evidence of linkage to 17q for this family is overwhelming; the LOD score with the linked haplotype is over 6.0, despite the existence of three cases of breast cancer which appear to be sporadic, i.e., these cases share no part of the linked haplotype between Mfd15 and 42D6. These three sporadic cases were diagnosed with breast cancer at ages 46, 47, and 54. In smaller kindreds, sporadic cancers of this type greatly confound the analysis of linkage and the correct identification of key recombinants. The key recombinant in the 2082 kindred is a woman who developed ovarian cancer at age 45 whose mother and aunt had ovarian cancer at ages 58 and 66, respectively. She inherited the linked portion of the haplotype for both Mfd188 and 42D6 while inheriting unlinked alleles at Mfd15; this recombinant event placed BRCA1 distal to Mfd15.

K1901 is typical of early-onset breast cancer kindreds. The kindred contains 10 cases of breast cancer with a median age at diagnosis of 43.5 years of age; four cases were diagnosed under age 40. The LOD score for this kindred with the marker 42D6 is 1.5, resulting in a posterior probability of 17q-linkage of 0.96. Examination of haplotypes in this kindred identified a recombinant haplotype in an obligate male carrier and his affected daughter who was diagnosed with breast cancer at age 45. Their linked allele for marker Mfd15 differs from that found in all other cases in the kindred (except one case which could not be completely inferred from her children). The two haplotypes are identical for Mfd188 and 42D6. Accordingly, data from Kindred 1901 would also place the BRCA1 locus distal to Mfd15.

Kindred 2035 is similar to K1901 in disease phenotype. The median age of diagnosis for the eight cases of breast cancer in this kindred is 37. One case also had ovarian cancer at age 60. The breast cancer cases in this family descend from two sisters who were both unaffected with breast cancer until their death in the eighth decade. Each branch contains four cases of breast cancer with at least one case in each branch having markedly early onset. This kindred has a LOD score of 2.34 with Mfd15. The haplotypes segregating with breast cancer in the two branches share an identical

allele at Mfd15 but differ for the distal loci Mfd188 and nm23 (a marker typed as part of the consortium which is located just distal to 42D6, (Hall *et al.*, 1992). Although the two haplotypes are concordant for marker 42D6, it is likely that the alleles are shared identical by state (the same allele but derived from different ancestors), rather than identical by descent (derived from a common ancestor) since the shared allele is the second most common allele observed at this locus. By contrast the linked allele shared at Mfd15 has a frequency of 0.04. This is a key recombinant in our dataset as it is the sole recombinant in which BRCA1 segregated with the proximal portion of the haplotype, thus setting the distal boundary to the BRCA1 region. For this event not to be a key recombinant requires that a second mutant BRCA1 gene be present in a spouse marrying into the kindred who also shares the rare Mfd15 allele segregating with breast cancer in both branches of the kindred. This event has a probability of less than one in a thousand. The evidence from this kindred therefore placed the BRCA1 locus proximal to Mfd188.

EXAMPLE 3

Creation of a Fine Structure Map and Refinement of the BRCA1 Region to Mfd191-Mfd188 using Additional STR Polymorphisms

In order to improve the characterization of our recombinants and define closer flanking markers, a dense map of this relatively small region on chromosome 17q was required. The chromosome 17 workshop has produced a consensus map of this region based on a combination of genetic and physical mapping studies (Fain, 1992). This map contains both highly polymorphic STR polymorphisms, and a number of nonpolymorphic expressed genes. Because this map did not give details on the evidence for this order nor give any measure of local support for inversions in the order of adjacent loci, we viewed it as a rough guide for obtaining resources to be used for the development of new markers and construction of our own detailed genetic and physical map of a small region containing BRCA1. Our approach was to analyze existing STR markers provided by other investigators and any newly developed markers from our laboratory with respect to both a panel of meiotic (genetic) breakpoints identified using DNA from the CEPH reference families and a panel of somatic cell hybrids (physical breakpoints) constructed for this region. These markers included 26C2 developed in our laboratory which maps proximal to Mfd15, Mfd191 (provided by James Weber), THRA1 (Futreal *et al.* 1992), and three polymorphisms kindly provided to us by Dr. Donald Black, NM23 (Hall *et al.* 1992), SCG40 (D17S181), and 6C1 (D17S293).

Genetic localization of markers. In order to localize new markers genetically within the region of interest, we have identified a number of key meiotic breakpoints within the region, both in the CEPH reference panel and in our large breast cancer kindred (K2082). Given the small genetic distance in this region, they are likely to be only a relatively small set of recombinants which can be used for this purpose, and they are likely to group markers into sets. The orders of the markers within each set can only be determined by physical mapping. However the number of genotypings necessary to position a new marker is minimized. These breakpoints are illustrated in Tables 3 and 4. Using this approach we were able to genetically order the markers THRA1, 6C1, SCG40, and Mfd191. As can be seen from Tables 3 and 4, THRA1 and MFD191 both map inside the Mfd15--Mfd188 region we had previously identified as containing the BRCA1 locus. In Tables 3 and 4, M/P indicates a maternal or paternal recombinant. A "1" indicates inherited allele is of grandpaternal origin, while a "0" indicates grandmaternal origin, and "-" indicates that the locus was untyped or uninformative.

TABLE 3
CEPH Recombinants

<u>Family</u>	<u>ID</u>	<u>M/P</u>	<u>Mfd15</u>	<u>THRA1</u>	<u>Mfd191</u>	<u>Mfd188</u>	<u>SCG40</u>	<u>6C1</u>	<u>42D6</u>
13292	4	M	1	1	1	0	0	0	0
13294	4	M	1	1	1	0	0	0	0
13294	6	M	0	0	1	1	-	-	-
1334	3	M	1	1	1	1	1	0	0
1333	4	M	1	1	1	0	-	-	0
1333	6	M	0	0	1	1	-	-	1
1333	8	P	1	0	0	0	-	-	0
1377	8	M	0	-	0	0	0	0	1

-50-

TABLE 4

Kindred 2082 Recombinants

<u>Family</u>	<u>ID</u>	<u>M/P</u>	<u>Mfd15</u>	<u>Mfd191</u>	<u>Mfd188</u>	<u>SCG40</u>	<u>6C1</u>	<u>42D6</u>
75		M	0	1	1	1	-	-
63		M	0	0	1	1	-	1
125		M	1	1	1	0	-	0
40		M	1	1	0	0	-	0

-51-

Analysis of markers Mfd15, Mfd188, Mfd191, and THRA1 in our recombinant families.

Mfd15, Mfd188, Mfd191 and THRA1 were typed in our recombinant families and examined for additional information to localize the BRCA1 locus. In kindred 1901, the Mfd15 recombinant was recombinant for THRA1 but uninformative for Mfd191, thus placing BRCA1 distal to THRA1. In
5 K2082, the recombinant with Mfd15 also was recombinant with Mfd191, thus placing the BRCA1 locus distal to Mfd191 (Goldgar *et al.*, 1994). Examination of THRA1 and Mfd191 in kindred K2035 yielded no further localization information as the two branches were concordant for both markers. However, SCG40 and 6C1 both displayed the same pattern as Mfd188, thus increasing our confidence in the localization information provided by the Mfd188 recombinant in this family. The
10 BRCA1 locus, or at least a portion of it, therefore lies within an interval bounded by Mfd191 on the proximal side and Mfd188 on the distal side.

EXAMPLE 4

Development of Genetic and Physical
Resources in the Region of Interest

15 To increase the number of highly polymorphic loci in the Mfd191-Mfd188 region, we developed a number of STR markers in our laboratory from cosmids and YACs which physically map to the region. These markers allowed us to further refine the region.

STSs were identified from genes known to be in the desired region to identify YACs which contained these loci, which were then used to identify subclones in cosmids P1s or BAC. These
20 subclones were then screened for the presence of a CA tandem repeat using a (CA)_n oligonucleotide (Pharmacia). Clones with a strong signal were selected preferentially, since they were more likely to represent CA-repeats which have a large number of repeats and/or are of near-perfect fidelity to the (CA)_n pattern. Both of these characteristics are known to increase the probability of polymorphism (Weber, 1990). These clones were sequenced directly from the vector to locate the
25 repeat. We obtained a unique sequence on one side of the CA-repeat by using one of a set of possible primers complementary to the end of a CA-repeat, such as (GT)₁₀T. Based on this unique sequence, a primer was made to sequence back across the repeat in the other direction, yielding a unique sequence for design of a second primer flanking the CA-repeat. STRs were then screened for polymorphism on a small group of unrelated individuals and tested against the hybrid panel to
30 confirm their physical localization. New markers which satisfied these criteria were then typed in a

set of 40 unrelated individuals from the Utah and CEPH families to obtain allele frequencies appropriate for the study population. Many of the other markers reported in this study were tested in a smaller group of CEPH unrelated individuals to obtain similarly appropriate allele frequencies.

5 Using the procedure described above, a total of eight polymorphic STRs were found from these
YACS. Of the loci identified in this manner, four were both polymorphic and localized to the
BRCA1 region. Four markers did not localize to chromosome 17, reflecting the chimeric nature of
the YACs used. The four markers which were in the region were denoted AA1, ED2, 4-7, and
YM29. AA1 and ED2 were developed from YACs positive for the RNU2 gene, 4-7 from an EPB3
YAC and YM29 from a cosmid which localized to the region by the hybrid panel. A description of
10 the number of alleles, heterozygosity and source of these four and all other STR polymorphisms
analyzed in the breast cancer kindreds is given below in Table 5.

TABLE 5

Polymorphic Short Tandem Repeat Markers Used
for Fine Structure Mapping of the BRCA1 Locus

Clone	Gene	Na**	Hetero- zygosity	Allele* Frequency (%)					
				1	2	3	4	5	6
Mfd15	D17S250	10	0.82	26	22	15	7	7	23
THRA1	THRA1	5							
Mfd191	D17S776	7	0.55	48	20	11	7	7	7
ED2	D17S1327	12	0.55	62	9	8	5	5	11
AA1	D17S1326	7	0.83	28	28	25	8	6	5
CA375	D17S184	10	0.75	26	15	11	9	9	20
4-7	D17S1183	9	0.50	63	15	8	6	4	4
YM29	--	9	0.62	42	24	12	7	7	8
Mfd188	D17S579	12	0.92	33	18	8	8	8	25
SCG40	D17S181	14	0.90	20	18	18	10	8	35
42D6	D17S588	11	0.86	21	17	11	10	9	32
6C1	D17S293	7	0.75	30	30	11	11	9	9
Z109	D17S750	9	0.70	33	27	7	7	7	19
tdj1475	D17S1321	13	0.84	21	16	11	11	8	33
CF4	D17S1320	6	0.63	50	27	9	7	4	3
tdj1239	D17S1328	10	0.80	86	10	9	7	4	14
U5	D17S1325	13	0.83	19	16	12	10	9	34

* Allele codes 1-5 are listed in decreasing frequency; allele numbers do not correspond to fragment sizes. Allele 6 frequency is the joint frequency of all other alleles for each locus.

** Number of alleles seen in the genetically independent DNA samples used for calculating allele frequencies.

The four STR polymorphisms which mapped physically to the region (4-7, ED2, AA1, YM29) were analyzed in the meiotic, breakpoint panel shown initially in Tables 3 and 4. Tables 6 and 7 contain the relevant CEPH data and Kindred 2082 data for localization of these four markers. In the tables, M/P indicates a maternal or paternal recombinant. A "1" indicates inherited allele is of grandpaternal origin, while a "0" indicates grandmaternal origin, and "-" indicates that the locus was untyped or uninformative.

5

TABLE 6

Key Recombinants Used for Genetic
Ordering of New STR Loci Developed in
Our Laboratory Within the BRCA1 Region of 17q

<u>CEPH</u> <u>Family</u>	<u>ID</u>	<u>M/P</u>	<u>Mfd15</u>	<u>THRA1</u>	<u>Mfd191</u>	<u>ED2</u>	<u>AA1</u>	<u>Z109</u>	<u>4-7</u>	<u>YM29</u>	<u>Mfd188</u>	<u>SCG40</u>	<u>42D6</u>
13292	4	M	1	1	1	1	1	0	0	0	0	0	0
13294	4	M	1	0	0	-	0	-	-	-	0	-	-
13294	6	M	0	0	1	-	1	-	-	-	1	-	-
1333	4	M	1	1	1	-	0	-	-	0	0	-	0
1333	6	M	0	0	1	-	1	-	-	1	1	-	1
1333	3	M	0	0	1	-	-	-	1	1	1	-	1

-56-

TABLE 7
Kindred 2082 Recombinants

<u>ID</u>	<u>M/P</u>	<u>Mfd15</u>	<u>Mfd191</u>	<u>ED2</u>	<u>AA1</u>	<u>4-7</u>	<u>YM29</u>	<u>Mfd188</u>	<u>SCG40</u>	<u>42D6</u>
63	M	0	0	1	-	1	1	1	1	1
125	M	1	1	1	-	1	1	1	0	0
40	M	1	1	0	-	0	-	0	0	0
22	P	0	0	1	1	1	1	1	1	1

-57-

From CEPH 1333-04, we see that AA1 and YM29 must lie distal to Mfd191. From 13292, it can be inferred that both AA1 and ED2 are proximal to 4-7, YM29, and Mfd188. The recombinants found in K2082 provide some additional ordering information. Three independent observations (individual numbers 22, 40, & 63) place AA1, ED2, 4-7, and YM29, and Mfd188 distal to Mfd191, while ID 125 places 4-7, YM29, and Mfd188 proximal to SCG40. No genetic information on the relative ordering within the two clusters of markers AA1/ED2 and 4-7/YM29/Mfd188 was obtained from the genetic recombinant analysis. Although ordering loci with respect to hybrids which are known to contain "holes" in which small pieces of interstitial human DNA may be missing is problematic, the hybrid patterns indicate that 4-7 lies above both YM29 and Mfd188.

EXAMPLE 5

Genetic Analyses of Breast Cancer Kindreds with Markers AA1, 4-7, ED2, and YM29

In addition to the three kindreds containing key recombinants which have been discussed previously, kindred K2039 was shown through analysis of the newly developed STR markers to be linked to the region and to contain a useful recombinant.

Table 8 defines the haplotypes (shown in coded form) of the kindreds in terms of specific marker alleles at each locus and their respective frequencies. In Table 8, alleles are listed in descending order of frequency; frequencies of alleles 1-5 for each locus are given in Table 5. Haplotypes coded H are BRCA1 associated haplotypes, P designates a partial H haplotype, and an R indicates an observable recombinant haplotype. As evident in Table 8, not all kindreds were typed for all markers; moreover, not all individuals within a kindred were typed for an identical set of markers, especially in K2082. With one exception, only haplotypes inherited from affected or at-risk kindred members are shown; haplotypes from spouses marrying into the kindred are not described. Thus in a given sibship, the appearance of haplotypes X and Y indicates that both haplotypes from the affected/at-risk individual were seen and neither was a breast cancer associated haplotype.

TABLE 8

Breast Cancer Linked Haplotypes
Found in the Three Kindreds

Kin.	HAP	Mfd 15	THRA1	Mfd 191	tdj 1475	ED2	AA1	Z109	CA375	4-7	YM29	Mfd 188	SCG40	6C1	42D6
1901	H1	1	5	5	3	1	4	NI	NI	1	1	3	NI	NI	1
	R2	9	2	5	6	1	4	NI	NI	1	1	3	NI	NI	1
2082	H1	3	NI	4	6	6	1	NI	NI	2	1	4	2	NI	1
	P1	3	NI	4	NI	NI	NI	NI	NI	NI	NI	4	2	NI	1
	P2	3	NI	NI	NI	NI	NI	NI	NI	NI	NI	4	NI	NI	NI
	R1	6	NI	1	5	6	1	NI	NI	2	1	4	2	NI	1
	R2	6	NI	4	6	6	1	NI	NI	2	1	4	2	NI	1
	R3	3	NI	4	NI	6	1	NI	NI	2	1	4	1	NI	7
	R4	7	NI	1	NI	1	5	NI	NI	4	6	1	2	NI	1
	R5	3	NI	4	NI	NI	NI	NI	NI	NI	2	1	NI	NI	NI
	R6	3	NI	4	3	1	2	NI	NI	1	2	2	6	NI	6
	R7	3	NI	4	3	7	1	NI	NI	1	1	3	7	NI	4
2035	H1	8	2	1	NI	5	1	1	4	3	1	6	8	2	4
	H2	8	2	1	NI	5	1	1	2	1	1	2	3	1	4
	R2	8	2	1	NI	5	1	1	2	1	1	2	3	6	1

-59-

In kindred K1901, the new markers showed no observable recombination with breast cancer susceptibility, indicating that the recombination event in this kindred most likely took place between THRA1 and ED2. Thus, no new BRCA1 localization information was obtained based upon studying the four new markers in this kindred. In kindred 2082 the key recombinant individual has inherited the linked alleles for ED2, 4-7, AA1, and YM29, and was recombinant for tdj1474 indicating that the recombination event occurred in this individual between tdj1474 and ED2/AA1.

There are three haplotypes of interest in kindred K2035, H1, H2, and R2 shown in Table 8. H1 is present in the four cases and one obligate male carrier descendant from individual 17 while H2 is present or inferred in two cases and two obligate male carriers in descendants of Individual 10. R2 is identical to H2 for loci between and including Mfd15 and SCG40, but has recombined between SCG40 and 42D6. Since we have established that BRCA1 is proximal to 42D6, this H2/R2 difference adds no further localization information. H1 and R2 share an identical allele at Mfd15, THRA1, AA1, and ED2 but differ for loci presumed distal to ED2, i.e., 4-7, Mfd188, SCG40, and 6C1. Although the two haplotypes are concordant for the 5th allele for marker YM29, a marker which maps physically between 4-7 and Mfd188, it is likely that the alleles are shared identical by state rather than identical by descent since this allele is the most common allele at this locus with a frequency estimated in CEPH parents of 0.42. By contrast, the linked alleles shared at the Mfd15 and ED2 loci have frequencies of 0.04 and 0.09, respectively. They also share more common alleles at Mfd191 (frequency = 0.52), THRA1, and AA1 (frequency = 0.28). This is the key recombinant in the set as it is the sole recombinant in which breast cancer segregated with the proximal portion of the haplotype, thus setting the distal boundary. The evidence from this kindred therefore places the BRCA1 locus proximal to 4-7.

The recombination event in kindred 2082 which places BRCA1 distal to tdj1474 is the only one of the four events described which can be directly inferred; that is, the affected mother's genotype can be inferred from her spouse and offspring, and the recombinant haplotype can be seen in her affected daughter. In this family the odds in favor of affected individuals carrying BRCA1 susceptibility alleles are extremely high; the only possible interpretations of the data are that BRCA1 is distal to Mfd191 or alternatively that the purported recombinant is a sporadic case of ovarian cancer at age 44. Rather than a directly observable or inferred recombinant, interpretation of kindred 2035 depends on the observation of distinct 17q-haplotypes segregating in different and sometimes distantly related branches of the kindred. The observation that portions of these haplotypes have alleles in common for some markers while they differ at other markers places the BRCA1 locus in

the shared region. The confidence in this placement depends on several factors: the relationship between the individuals carrying the respective haplotypes, the frequency of the shared allele, the certainty with which the haplotypes can be shown to segregate with the BRCA1 locus, and the density of the markers in the region which define the haplotype. In the case of kindred 2035, the two branches are closely related, and each branch has a number of early onset cases which carry the respective haplotype. While two of the shared alleles are common, (Mfd191, THRA1), the estimated frequencies of the shared alleles at Mfd15, AA1, and ED2 are 0.04, 0.28, and 0.09, respectively. It is therefore highly likely that these alleles are identical by descent (derived from a common ancestor) rather than identical by state (the same allele but derived from the general population).

EXAMPLE 6

Refined Physical Mapping Studies Place the BRCA1 Gene in a Region Flanked by tdj11474 and U5R

Since its initial localization to chromosome 17q in 1990 (Hall *et al.* 1990) a great deal of effort has gone into localizing the BRCA1 gene to a region small enough to allow implementation of effective positional cloning strategies to isolate the gene. The BRCA1 locus was first localized to the interval Mfd15 (D17S250) - 42D6 (D17S588) by multipoint linkage analysis (Easton *et al.* 1993) in the collaborative Breast Cancer Linkage Consortium dataset consisting of 214 families collected worldwide. Subsequent refinements of the localization have been based upon individual recombinant events in specific families. The region THRA1 - D17S183 was defined by Bowcock *et al.*, 1993; and the region THRA1 - D17S78 was defined by Simard *et al.*, 1993.

We further showed that the BRCA1 locus must lie distal to the marker Mfd191 (D17S776) (Goldgar *et al.* 1994). This marker is known to lie distal to THRA1 and RARA. The smallest published region for the BRCA1 locus is thus between D17S776 and D17S78. This region still contains approximately 1.5 million bases of DNA, making the isolation and testing of all genes in the region a very difficult task. We have therefore undertaken the tasks of constructing a physical map of the region, isolating a set of polymorphic STR markers located in the region, and analyzing these new markers in a set of informative families to refine the location of the BRCA1 gene to a manageable interval.

Four families provide important genetic evidence for localization of BRCA1 to a sufficiently small region for the application of positional cloning strategies. Two families (K2082, K1901) provide data relating to the proximal boundary for BRCA1 and the other two (K2035, K1813) fix the distal boundary. These families are discussed in detail below. A total of 15 Short Tandem Repeat markers assayable by PCR were used to refine this localization in the families studied. These markers include DS17S7654, DS17S975, tdj1474, and tdj1239. Primer sequences for these markers are provided in SEQ ID NO:3 and SEQ ID NO:4 for DS17S754; in SEQ ID NO:5 and SEQ ID NO:6 for DS17S975; in SEQ ID NO:7 and SEQ ID NO:8 for tdj1474; and, in SEQ ID NO:9 and SEQ ID NO:10 for tdj1239.

Kindred 2082

Kindred 2082 is the largest BRCA1-linked breast/ovarian cancer family studied to date. It has a Lod score of 8.6, providing unequivocal evidence for 17q linkage. This family has been previously described and shown to contain a critical recombinant placing BRCA1 distal to MFD191 (D17S776). This recombinant occurred in a woman diagnosed with ovarian cancer at age 45 whose mother had ovarian cancer at age 63. The affected mother was deceased; however, from her children, she could be inferred to have the linked haplotype present in the 30 other linked cases in the family in the region between Mfd15 and Mfd188. Her affected daughter received the linked allele at the loci ED2, 4-7, and Mfd188, but received the allele on the non-BRCA1 chromosome at Mfd15 and Mfd191. In order to further localize this recombination breakpoint, we tested the key members of this family for the following markers derived from physical mapping resources: tdj1474, tdj1239, CF4, D17S855. For the markers tdj1474 and CF4, the affected daughter did not receive the linked allele. For the STR locus tdj1239, however, the mother could be inferred to be informative and her daughter did receive the BRCA1-associated allele. D17S855 was not informative in this family. Based on this analysis, the order is 17q centomere - Mfd191 - 17HSD - CF4 - tdj1474 - tdj1239 - D17S855 - ED2 - 4-7 - Mfd188 - 17q telomere. The recombinant described above therefore places BRCA1 distal to tdj1474, and the breakpoint is localized to the interval between tdj1474 and tdj1239. The only alternative explanation for the data in this family other than that of BRCA1 being located distal to tdj1474, is that the ovarian cancer present in the recombinant individual is caused by reasons independent of the BRCA1 gene. Given that ovarian cancer diagnosed before age 50 is rare, this alternate explanation is exceedingly unlikely.

Kindred 1901

Kindred 1901 is an early-onset breast cancer family with 7 cases of breast cancer diagnosed before 50, 4 of which were diagnosed before age 40. In addition, there were three cases of breast cancer diagnosed between the ages of 50 and 70. One case of breast cancer also had ovarian cancer at age 61. This family currently has a Lod score of 1.5 with D17S855. Given this linkage evidence and the presence of at least one ovarian cancer case, this family has a posterior probability of being due to BRCA1 of over 0.99. In this family, the recombination comes from the fact that an individual who is the brother of the ovarian cancer case from which the majority of the other cases descend, only shares a portion of the haplotype which is cosegregating with the other cases in the family. However, he passed this partial haplotype to his daughter who developed breast cancer at age 44. If this case is due to the BRCA1 gene, then only the part of the haplotype shared between this brother and his sister can contain the BRCA1 gene. The difficulty in interpretation of this kind of information is that while one can be sure of the markers which are not shared and therefore recombinant, markers which are concordant can either be shared because they are non-recombinant, or because their parent was homozygous. Without the parental genotypic data it is impossible to discriminate between these alternatives. Inspection of the haplotype in K1901, shows that he does not share the linked allele at Mfd15 (D17S250), THRA1, CF4 (D17S1320), and tdj1474 (D17S1321). He does share the linked allele at Mfd191 (D17S776), ED2 (D17S1327), tdj1239 (D17S1328), and Mfd188 (D17S579). Although the allele shared at Mfd191 is relatively rare (0.07), we would presume that the parent was homozygous since they are recombinant with markers located nearby on either side, and a double recombination event in this region would be extremely unlikely. Thus the evidence in this family would also place the BRCA1 locus distal to tdj1474. However, the lower limit of this breakpoint is impossible to determine without parental genotype information. It is intriguing that the key recombinant breakpoint in this family confirms the result in Kindred 2082. As before, the localization information in this family is only meaningful if the breast cancer was due to the BRCA1 gene. However, her relatively early age at diagnosis (44) makes this seem very likely since the risk of breast cancer before age 45 in the general population is low (approximately 1%).

Kindred 2035

This family is similar to K1901 in that the information on the critical recombinant events is not directly observed but is inferred from the observation that the two haplotypes which are cosegregating with the early onset breast cancer in the two branches of the family appear identical

for markers located in the proximal portion of the 17q BRCA1 region but differ at more distal loci. Each of these two haplotypes occurs in at least four cases of early-onset or bilateral breast cancer. The overall LOD score with ED2 in this family is 2.2, and considering that there is a case of ovarian cancer in the family (indicating a prior probability of BRCA1 linkage of 80%), the resulting posterior probability that this family is linked to BRCA1 is 0.998. The haplotypes are identical for the markers Mfd15, THRA1, Mfd191, ED2, AA1, D17S858 and D17S902. The common allele at Mfd15 and ED2 are both quite rare, indicating that this haplotype is shared identical by descent. The haplotypes are discordant, however, for CA375, 4-7, and Mfd188, and several more distal markers. This indicates that the BRCA1 locus must lie above the marker CA-375. This marker is located approximately 50 kb below D17S78, so it serves primarily as additional confirmation of this previous lower boundary as reported in Narod *et al.* (1993).

Kindred 1813

Kindred 1813 is a small family studied by one of our collaborators with four cases of breast cancer diagnosed under the age of 40 whose mother had breast cancer diagnosed at age 45 and ovarian cancer at age 61. This situation is somewhat complicated by the fact the four cases appear to have three different fathers, only one of whom has been genotyped. However, by typing a number of different markers in the BRCA1 region as well as highly polymorphic markers elsewhere in the genome, the paternity of all children in the family has been determined with a high degree of certainty. This family yields a maximum multipoint lod score of 0.60 with 17q markers and, given that there is at least one case of ovarian cancer, results in a posterior probability of being a BRCA1 linked family of 0.93. This family contains a directly observable recombination event in individual 18 (see Figure 5 in Smard *et al.*, *Human Mol. Genet.* 2:1193-1199 (1993)), who developed breast cancer at age 34. The genotype of her affected mother at the relevant 17q loci can be inferred from her genotypes, her affected sister's genotypes, and the genotypes of three other unaffected siblings. Individual 18 inherits the BRCA1-linked alleles for the following loci: Mfd15, THRA1, D17S800, D17S855, AA1, and D17S931. However, for markers below D17S931, i.e., U5R, vrs31, D17S858, and D17S579, she has inherited the alleles located on the non-disease bearing chromosome. The evidence from this family therefore would place the BRCA1 locus proximal to the marker U5R. Because of her early age at diagnosis (34) it is extremely unlikely that the recombinant individual's cancer is not due to the gene responsible for the other cases of breast/ovarian cancer in this family; the uncertainty in this family comes from our somewhat smaller amount of evidence that breast

cancer in this family is due to BRCA1 rather than a second, as yet unmapped, breast cancer susceptibility locus.

Size of the region containing BRCA1

Based on the genetic data described in detail above, the BRCA1 locus must lie in the interval between the markers tdj1474 and U5R, both of which were isolated in our laboratory. Based upon the physical maps shown in Figure 2 and 3, we can try to estimate the physical distance between these two loci. It takes approximately 14 P1 clones with an average insert size of approximately 80 kb to span the region. However, because all of these P1s overlap to some unknown degree, the physical region is most likely much smaller than 14 times 80 kb.. Based on restriction maps of the clones covering the region, we estimate the size of the region containing BRCA1 to be approximately 650 kb.

EXAMPLE 7

Identification of Candidate cDNA Clones for the BRCA1 Locus by Genomic Analysis of the Contig Region

Complete screen of the plausible region The first method to identify candidate cDNAs, although labor intensive, used known techniques. The method comprised the screening of cosmids and P1 and BAC clones in the contig to identify putative coding sequences. The clones containing putative coding sequences were then used as probes on filters of cDNA libraries to identify candidate cDNA clones for future analysis. The clones were screened for putative coding sequences by either of two methods.

Zoo blots. The first method for identifying putative coding sequences is by screening the cosmid and P1 clones for sequences conserved through evolution across several species. This technique is referred to as "zoo blot analysis" and is described by Monaco, 1986. Specifically, DNA from cow, chicken, pig, mouse and rat are digested with the restriction enzymes EcoRI and HindIII (8 µg of DNA per enzyme). The digested DNAs are separated overnight on an 0.7% gel at 20 volts for 16 hours (14 cm gel), and the DNA transferred to Nylon membranes using standard Southern blot techniques. For example, the zoo blot filter is treated at 65°C in 0.1 x SSC, 0.5% SDS, and 0.2M Tris, pH 8.0, for 30 minutes and then blocked overnight at 42°C in 5x SSC, 10% PEG 8000, 20 mM

NaPO₄ pH 6.8, 100 µg/ml Salmon Sperm DNA, 1x Denhardt's, 50% formamide, 0.1% SDS, and 2 µg/ml cot-1 DNA.

The cosmid and Pl clones to be analyzed are digested with a restriction enzyme to release the human DNA from the vector DNA. The DNA is separated on a 14 cm, 0.5% agarose gel run overnight at 20 volts for 16 hours. The human DNA bands are cut out of the gel and electroeluted from the gel wedge at 100 volts for at least two hours in 0.5x Tris Acetate buffer (Maniatis *et al.*, 1989). The eluted Not I digested DNA (~15 kb to 25 kb) is then digested with EcoRI restriction enzyme to give smaller fragments (~0.5 kb to 5.0 kb) which melt apart more easily for the next step of labeling the DNA with radionucleotides. The DNA fragments are labeled by means of the hexamer random prime labeling method (Boehringer-Mannheim, Cat. #1004760). The labeled DNA is spermine precipitated (add 100 µl TE, 5 µl 0.1 M spermine, and 5 µl of 10 mg/ml salmon sperm DNA) to remove unincorporated radionucleotides. The labeled DNA is then resuspended in 100 µl TE, 0.5 M NaCl at 65°C for 5 minutes and then blocked with Human Cot-1 DNA for 2-4 hrs. as per the manufacturer's instructions (Gibco/BRL, Cat. #5279SA). The Cot-1 blocked probe is incubated on the zoo blot filters in the blocking solution overnight at 42°C. The filters are washed for 30 minutes at room temperature in 2 x SSC, 0.1% SDS, and then in the same buffer for 30 minutes at 55°C. The filters are then exposed 1 to 3 days at -70°C to Kodak XAR-5 film with an intensifying screen. Thus, the zoo blots are hybridized with either the pool of Eco-R1 fragments from the insert, or each of the fragments individually. .

HTF island analysis. The second method for identifying cosmids to use as probes on the cDNA libraries is HTF island analysis. Since the pulsed-field map can reveal HTF islands, cosmids that map to these HTF island regions are analyzed with priority. HTF islands are segments of DNA which contain a very high frequency of unmethylated CpG dinucleotides (Tonolio *et al.*, 1990) and are revealed by the clustering of restriction sites of enzymes whose recognition sequences include CpG dinucleotides. Enzymes known to be useful in HTF-island analysis are AscI, NotI, BssHII, EagI, SacII, NaeI, NarI, SmaI, and MluI (Anand, 1992). A pulsed-field map has been created using the enzymes NotI, NruI, EagI, SacII, and SalI, and two HTF islands were found. These islands are located in the distal end of the region, one being distal to the GP2B locus, and the other being proximal to the same locus, both outside the BRCA1 region. The cosmids derived from the YACs that cover these two locations are analyzed to identify those that contain these restriction sites, and thus the HTF islands.

cDNA screening. Those clones that contain HTF islands or show hybridization to other species DNA besides human are likely to contain coding sequences. The human DNA from these clones is isolated as whole insert or as EcoRI fragments and labeled as described above. The labeled DNA is used to screen filters of various cDNA libraries under the same conditions as the zoo blots except
5 that the cDNA filters undergo a more stringent wash of 0.1 x SSC, 0.1% SDS at 65°C for 30 minutes twice.

Most of the cDNA libraries used to date in our studies (libraries from normal breast tissue, breast tissue from a woman in her eighth month of pregnancy and a breast malignancy) were prepared at Clontech, Inc. The cDNA library generated from breast tissue of an 8 month pregnant
10 woman is available from Clontech (Cat. #HL1037a) in the Lambda gt-10 vector, and is grown in C600Hfl bacterial host cells. Normal breast tissue and malignant breast tissue samples were isolated from a 37 year old Caucasian female and one-gram of each tissue was sent to Clontech for mRNA processing and cDNA library construction. The later two libraries were generated using both random and oligo-dT priming, with size selection of the final products which were then cloned into the
15 Lambda Zap II vector, and grown in XL1-blue strain of bacteria as described by the manufacturer. Additional tissue-specific cDNA libraries include human fetal rain (Stratagene, Cat. 936206), human testing (Clontech Cat. HL3024), human thymus (Clontech Cat. HL1127n), human brain (Clontech Cat. HL11810), human placenta (Clontech Cat 1075b), and human skeletal muscle (Clontech Cat. HL1124b).

20 The cDNA libraries are plated with their host cells on NZCYM plates, and filter lifts are made in duplicate from each plate as per Maniatis *et al.*(1989). Insert (human) DNA from the candidate genomic clones is purified and radioactively labeled to high specific activity. The radioactive DNA is then hybridized to the cDNA filters to identify those cDNAs which correspond to genes located within the candidate cosmid clone. cDNAs identified by this method are picked, replated, and
25 screened again with the labeled clone insert or its derived EcoRI fragment DNA to verify their positive status. Clones that are positive after this second round of screening are then grown up and their DNA purified for Southern blot analysis and sequencing. Clones are either purified as plasmid through *in vivo* excision of the plasmid from the Lambda vector as described in the protocols from the manufacturers, or isolated from the Lambda vector as a restriction fragment and subcloned into
30 plasmid vector.

The Southern blot analysis is performed in duplicate, one using the original genomic insert DNA as a probe to verify that cDNA insert contains hybridizing sequences. The second blot is

hybridized with cDNA insert DNA from the largest cDNA clone to identify which clones represent the same gene. All cDNAs which hybridize with the genomic clone and are unique are sequenced and the DNA analyzed to determine if the sequences represent known or unique genes. All cDNA clones which appear to be unique will be further analyzed as candidate BRCA1 loci. Specifically, the clones will be hybridized to Northern blots to look for breast specific expression and differential expression in normal versus breast tumor RNAs. They will also be analyzed by PCR on clones in the BRCA1 region to verify their location. To map the extent of the locus, full length cDNAs are isolated and their sequences used as PCR probes on the YACs and the clones surrounding and including the original identifying clones. Intron-exon boundaries are then further defined through sequence analysis.

We have screened the normal breast, 8 month pregnant breast and fetal brain cDNA libraries with zoo blot-positive Eco RI fragments from cosmid BAC and P1 clones in the region. Potential BRCA1 cDNA clones were identified among the three libraries. Clones were picked, replated, and screened again with the original probe to verify that they were positive.

Analysis of hybrid-selected cDNA. cDNA fragments obtained from direct selection were checked by Southern blot hybridization against the probe DNA to verify that they originated from the contig. Those that passed this test were sequenced in their entirety. The set of DNA sequences obtained in this way were then checked against each other to find independent clones that overlapped. For example, the clones 694-65, 1240-1 and 1240-33 were obtained independently and subsequently shown to derive from the same contiguous cDNA sequence which has been named EST:489:1.

Analysis of candidate clones. One or more of the candidate genes generated from above are sequenced and the information used for identification and classification of each expressed gene. The DNA sequences are compared to known genes by nucleotide sequence comparisons and by translation in all frames followed by a comparison with known amino acid sequences. This is accomplished using Genetic Data Environment (GDE) version 2.2 software and the Basic Local Alignment Search Tool (Blast) series of client/server software packages (e.g., BLASTN 1.3.13MP), for sequence comparison against both local and remote sequence databases (e.g., GenBank), running on Sun SPARC workstations. Sequences reconstructed from collections of cDNA clones identified with the cosmids and PIs have been generated. All candidate genes that represented new sequences were analyzed further to test their candidacy for the putative BRCA1 locus.

Mutation screening. To screen for mutations in the affected pedigrees, two different approaches are followed. First, genomic DNA isolated from family members known to carry the susceptibility

allele of BRCA1 is used as a template for amplification of candidate gene sequences by PCR. If the PCR primers flank or overlap an intron/exon boundary, the amplified fragment will be larger than predicted from the cDNA sequence or will not be present in the amplified mixture. By a combination of such amplification experiments and sequencing of Pl, BAC or cosmid clones using the set of designed primers it is possible to establish the intron/exon structure and ultimately obtain the DNA sequences of genomic DNA from the pedigrees.

A second approach that is much more rapid if the intron/exon structure of the candidate gene is complex involves sequencing fragments amplified from pedigree lymphocyte cDNA. cDNA synthesized from lymphocyte mRNA extracted from pedigree blood is used as a substrate for PCR amplification using the set of designed primers. If the candidate gene is expressed to a significant extent in lymphocytes, such experiments usually produce amplified fragments that can be sequenced directly without knowledge of intron/exon junctions.

The products of such sequencing reactions are analyzed by gel eletrophoresis to determine positions in the sequence that contain either mutations such as deletions or insertions, or base pair substitutions that cause amino acid changes or other detrimental effects.

Any sequence within the BRCA1 region that is expressed in breast is considered to be a candidate gene for BRCA1. Compelling evidence that a given candidate gene corresponds to BRCA1 comes from a demonstration that pedigree families contain defective alleles of the candidate.

EXAMPLE 8

Identification of BRCA1

Newly obtained cDNA sequences were checked against sequences in Genbank for similarities to known sequences. In the case of BRCA1 (SEQ ID NO:1), the predicted amino acid sequence (SEQ ID NO:2) of the longest open reading frame showed no significant homology with Genbank sequences. Screening of the Stratagene fetal brain cDNA library was used to find clones that apparently collectively contain the entire coding region of the BRCA1 gene. The cDNA sequence has a long open reading frame (1659 a.a.) ending with a polyadenylation signal (AATAAA) and a poly A tract. The genomic DNA sequences determined for BRCA1 is set forth in SEQ ID NO:11., in which the exons have been identified. The intorn/exon junctions are shown in Table 9.

TABLE 9

Exon Number	Base Position		Length	Intron Borders	
	5'	3'		5'	3'
e1	1	103	102	TGCTTGACTGTCTTACCAWACTGTAARCAG ^{12*}	GGTAAGGTCTCAGGTTTTTTTAAGTATTGCT ¹³
e2	104	3530	3426	GTGAGTACCTTGTTATTTNIGTATATTTCA ¹⁴	-na-
e3	3531	3624	93	-na-	-na-
e4	3625	3791	166	ATTTCTTGGTGCCATTTATCGTTTTTGAAG ¹⁵	GTATTGTTGGCCAAACACTGATATCTTAAG ¹⁶
e5	3792	3913	121	AGATTTGTTTTCTCATTCCATTTAAAGCAG ¹⁷	GTAAGAAACATCAATGTAAAGATGCTGTGG ¹⁸
e6	3914	4106	192	ATGGTTTTCTCCTTTTCATTTATCTTTCTAG ¹⁹	GTAATATTTTCATCTGCTGTATTGGARCAMA ²⁰
e7	4107	4149	42	-na-	-na-
e8	4150	4417	267	TTCCCNAGNAGNGGNANACCCYYTCYCYKR ²¹	GTGTATCCATATGTATCTCCCTAATGACTA ²²
e9	4418	4506	88	ATGATAATGGAATATTTGATTTAATTTTCAG ²³	GTATACCAAGAACCTTTACAGAATACCTTG ²⁴
e10	4507	4585	78	TAATCCTTTGAGTGKTCTCATTCTGCAGA ²⁵	KAAGKATAATACTATWTCTCCCCYCCTCCC ²⁶
e11	4586	4624	38	TAACCTGTCTTTTCTATGATCTCTTTAGGG ²⁷	GTAAGTACTTGATGTTACAACTAACCAGA ²⁸
e12	4625	4708	83	TATCCTGATGGGTTGTGTTTTGTTTTTTCAG ²⁹	GTAAAGCTCCCTCCCTCAAGTTGACAAAAA ³⁰
e13	4709	4898	189	-na-	-na-
e14	4899	5226	327	CTAATCTCTGCTTGTGTTCTCTGTCTCCAG ³¹	-na-

* Numbers in superscript refer to SEQ ID NOS.

Mutation screening of BRCA1. To screen BRCA1 for mutations in these kindreds, the 14 coding exons and associated splice junctions were amplified from genomic DNA samples and the DNA sequences were compared to the wild-type sequence. In other experiments, coding sequences were amplified from cDNA prepared from lymphocyte mRNA prior to DNA sequence analysis. 4/9 samples contained sequence variants. Kindred 2082 contained a nonsense mutation at 3330 in exon 2. Kindred 1910 contained a single nucleotide insertion (C) at 4656 in the 12th coding exon; kindred 2099 contained a missense mutation at 4714 in exon 13, resulting in a met->arg substitution. The frameshift mutation in kindred 1910 was likely disruptive to the function of the BRCA1 product as it resulted in an altered reading frame changing the last 108 bases of the coding sequence and prematurely terminating the protein. All sequence variants wre heterozygous. In one of the kindreds (2035), only a single nonsilent variant was detected in genomic DNA, . However, a conservative substitution (Ser->Gly) at nucleotide position 4222 in exon 8 was observed in this kindred to be heterozygous. Interestingly, in cDNA samples from one member of the kindreds this polymorphic site appeared homozygous, suggesting that mRNA from one of the alleles was absent. This analysis indicated that kindred may contain a mutation in BRCA1 that either prevents transcription, causes instability of BRCA1 mRNA, or causes aberrant splicing of the BRCA1 transcript. A missense mutation (Ala->Glu) was detected on Sloan-Kettering tumor sample 890808.1 at position 4513 in exon 10. This mutation was also seen in the germline of that patient and not in 100 other tumor samples. The tumor was tested and found to have LOH in the BRCA1 region.

20

TABLE 10
PREDISPOSING MUTATIONS

	<u>Kindred Number</u>	<u>Mutation</u>	<u>Coding Effect</u>	<u>Location*</u>
25	2082	C->T	Gln->Stop	3330
	1910	extra C	frameshift	4656
	2099	T->G	Met->Arg	4714
	2035	?	loss of transcript	detected by polymorphism at 4227
30	Tumor ID 890808.1	C->A	Ala->Glu	4513

* In Sequence ID NO:1

Further confirmation of the significance of the polymorphism comes from linkage tests in the kindreds. Both the C→T in 2082 and the additional C in 1910 were found to be linked to the chromosome carrying the BRCA1 haplotype. Neither of these mutations were seen in 90 normal controls. Initial screening found the G→T mutation in two distantly related carriers. This would mean that the mutation is on the chromosome carrying the BRCA1 haplotype. If the polymorphism causes a predisposition to cancer, it must be present on the predisposing chromosome and not on the homologous chromosome in the individuals tested. Finally, we expect to find other mutations in pedigrees and possibly in tumor DNA samples. Screening the remaining portion of the BRCA1 gene should reveal mutations in most, if not all, of the other BRCA1 kindreds. In addition, a breast tumor line from Sloan-Kettering was screened for a mutation. It was found that both the germline and the tumor did contain a mutation in BRCA1, a mutation of a C→A at nucleotide 4513.

Tissue Distribution of BRCA1 Expression. Northern blot analysis was performed with RNA isolated from a range of fetal and adult tissues and a radioactively labeled probe corresponding to position 3029 through 3327 of BRCA1. A relatively strong signal was observed in breast, testis, thymus, and fetal liver; a weaker signal was observed in spleen, ovary, small intestine, colon, and fetal heart, lung, and kidney.

Additionally, the distribution of BRCA transcript was determined by PCR using cDNA derived from a range of human tissues as template. Primers corresponding to positions 3029 and 3327 produced a PCR product using cDNA from breast, thymus, lymphocyte, ovary and spleen.

Industrial Utility

As previously described above, the present invention provides materials and methods for use in testing BRCA1 alleles of an individual and an interpretation of the normal or predisposing nature of the alleles. Individuals at higher than normal risk might modify their lifestyles appropriately. In the case of BRCA1, the most significant non-genetic risk factor is the protective effect of an early, full term pregnancy. Therefore, women at risk could consider early childbearing or a therapy designed to simulate the hormonal effects of an early full-term pregnancy. Women at high risk would also strive for early detection and would be more highly motivated to learn and practice breast self examination. Such women would also be highly motivated to have regular mammograms, perhaps starting at an earlier age than the general population. Ovarian screening could also be undertaken at greater frequency. Diagnostic methods based on sequence analysis of the BRCA1 locus

could also be applied to tumor detection and classification. Sequence analysis could be used to diagnose precursor lesions. With the evolution of the method and the accumulation of information about BRCA1 and other causative loci, it could become possible to separate cancers into benign and malignant.

5 Women with breast cancers may follow different surgical procedures if they are predisposed, and therefore likely to have additional cancers, than if they are not predisposed. Other therapies may be developed, using either peptides or small molecules (rational drug design). Peptides could be the missing gene product itself or a portion of the missing gene product. Alternatively, the therapeutic agent could be another molecule that mimics the deleterious gene's function, either a peptide or a
10 nonpeptidic molecule that seeks to counteract the deleterious effect of the inherited locus. The therapy could also be gene based, through introduction of a normal BRCA1 allele into individuals to make a protein which will counteract the effect of the deleterious allele. These gene therapies may take many forms and may be directed either toward preventing the tumor from forming, curing a cancer once it has occurred, or stopping a cancer from metastasizing.

15 It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variety of embodiments, only a few of which are disclosed herein. It will be apparent to the artisan that other embodiments exist and do not depart from the spirit of the invention. Thus, the described embodiments are illustrative and should not be construed as restrictive.

LIST OF REFERENCES

- Anand, R. (1992). *Techniques for the Analysis of Complex Genomes*, (Academic Press).
- Anderson, *et al.* (1980). *Proc. Natl. Acad. Sci. USA* **77**:5399-5403.
- Anderson, J.A., *et al.* (1992). *J. Otolaryngology* **21**:321.
- Anderson, D.E. (1992). *J. Natl. Cancer Inst.* **48**:1029-1034.
- Ausebel, F.M., *et al.* (1992). *Current Protocols in Molecular Biology*, (J. Wiley and Sons, N.Y.)
- Beaucage & Carruthers (1981). *Tetra. Letts.* **22**:1859-1862.
- Berkner, *et al.* (1988). *BioTechniques* **6**:616-629.
- Berkner (1992). *Curr. Top. Microbiol. Immunol.* **158**:39-61.
- Bishop, D.T., *et al.* (1988). *Genet. Epidemiol.* **5**:151-169.
- Bishop, D.T. and Gardner, E.J. (1980). In: Banbury Report 4: Cancer Incidence in Defined Populations (J. Cairns, J.L. Lyon, M. Skolnick, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 309-408.
- Botstein, *et al.* (1980). *Am. J. Hum. Genet.* **32**:314-331.
- Brandyopadhyay and Temin (1984). *Mol. Cell. Biol.* **4**:749-754.
- Breakfield and Geller (1987). *Mol. Neurobiol.* **1**:337-371.
- Brinster, *et al.* (1981). *Cell* **27**:223-231.
- Buchschacher and Panganiban (1992). *J. Virol.* **66**:2731-2739.
- Buckler, *et al.* (1991). *Proc. Natl. Acad. Sci. USA* **88**:4005-4009.
- Capecchi, M.R. (1989). *Science* **244**:1288.
- Cariello (1988). *Human Genetics* **42**:726.
- Claus, E., *et al.* (1991). *Am. J. Hum. Genet.* **48**:232-242.
- Conner, B.J., *et al.* (1983). *Proc. Nat. Acad. Sci. USA.* _____
- Constantini and Lacy (1981). *Nature* **294**:92-94.
- Cotten, *et al.* (1990). *Proc. Natl. Acad. Sci. USA* **87**:4033-4037.

- Cotton, *et al.* (1988). *Proc. Nat. Acad. Sci. USA* **85**:4397.
- Culver, *et al.* (1992). *Science* **256**:1550-1552.
- Curiel, *et al.* (1991). *Proc. Natl. Acad. Sci. USA* **88**:8850-8854.
- Curiel, *et al.* (1991). *Hum. Gene Ther.* **3**:147-154.
- Deutscher, M. (1990). *Meth. Enzymology* **182**, (Academic Press, San Diego, Cal.).
- Donehower, L.A., *et al.* (1992). *Nature* **356**:215.
- Easton, D., *et al.* (1993). *Am. J. Hum. Genet.* **52**:678-701.
- Erickson, J. *et al.*, (1990). *Science* **249**:527-533.
- Fain, P.R. (1992). *Cytogen. Cell Genet.* **60**:178.
- Felgner, *et al.* (1987). *Proc. Natl. Acad. Sci. USA* **84**:7413-7417.
- Fiers, *et al.* (1978). *Nature* **273**:113.
- Fink, *et al.* (1992). *Hum. Gene Ther.* **3**:11-19.
- Finkelstein, J., *et al.* (1990). *Genomics* **7**:167-172.
- Freese, *et al.* (1990). *Biochem. Pharmacol.* **40**:2189-2199.
- Friedman, T. (1991). In *Therapy for Genetic Diseases*, T. Friedman, ed., Oxford University Press, pp. 105-121.
- Futreal, A., *et al.* (1992). *Hum. Molec. Genet.* **1**:66.
- Futreal (1993). Ph.D. Thesis, University of North Carolina, Chapel Hill.
- Glover, D. (1985). *DNA Cloning, I and II*, (Oxford Press).
- Go, R.C.P., *et al.* (1983). *J. Natl. Cancer Inst.* **71**:455-461.
- Goding (1986). *Monoclonal Antibodies: Principles and Practice*, 2d ed. (Academic Press, N.Y.).
- Godowski, *et al.* (1988). *Science* **241**:812-816.
- Goldgar, D.E., *et al.* (1994). *J. Natl. Can. Inst.* **86**:3:200-209.
- Gordon, *et al.* (1980). *Proc. Natl. Acad. Sci. USA* **77**:7380-7384.
- Gorziglia and Kapikian (1992). *J. Virol.* **66**:4407-4412.

- Graham and van der Eb (1973). *Virology* **52**:456-467.
- Guthrie, G. & Fink, G.R. (1991). *Guide to Yeast Genetics and Molecular Biology*, (Academic Press).
- Hall, J.M., *et al.* (1992). *Am. J. Hum. Genet.* **50**:1235-1241.
- Hall, J.M., *et al.* (1990). *Science* **250**:1684-1689.
- Harlow & Lane (1988). Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Hasty, P., K., *et al.* (1991). *Nature* **350**:243.
- Helseth, *et al.* (1990). *J. Virol.* **64**:2416-2420.
- Hodgson, J. (1991). *Bio/Technology* **9**:19-21.
- Huse, *et al.* (1989). *Science* **246**:1275-1281.
- Innis *et al.* (1990). *PCR Protocols: A Guide to Methods and Applications*, (Academic Press, San Diego, Cal.).
- Jeffreys, *et al.* (1985). *Nature* **314**:67-73.
- Johnson, J.L., *et al.* (1994). *Mol. Cell Biol.* **14**:1956-63.
- Johnson, *et al.* (1992). *J. Virol.* **66**:2952-2965.
- Kamb, A. *et al.* (1994). *Science* **264**:436-440.
- Kandpal, *et al.* (1990). *Nucleic Acids Research* **18**:1789-1795.
- Kaneda, *et al.* (1989). *J. Biol. Chem.* **264**:12126-12129.
- Kanehisa (1984). *Nuc. Acids Res.* **12**:203-213.
- Kinszler, K.W., *et al.* (1991). *Science* **251**:1366-1370.
- Knudson, A.G. (1993). *Nature Genet.* **5**:103.
- Kubo, T., *et al.* (1988). *FEBS Lett.* **241**:119.
- Landegren, *et al.* (1988). *Science* **242**:229.
- Lim, *et al.* (1992). *Circulation* **83**:2007-2011.
- Lindsay, S., *et al.* (1987). *Nature* **327**:336-368.

- Litt, *et al.* (1989). *Am. J. Hum. Genet.* 44:397-401.
- Lovett, *et al.* (1991). *Proc. Natl. Acad. Sci. USA* 88:9628-9632.
- Madzak, *et al.* (1992). *J. Gen. Virol.* 73:1533-1536.
- Malkin, D., *et al.* (1990). *Science* 250:1233-1238.
- Maniatis. T., *et al.* (1989). *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Mann and Baltimore (1985). *J. Virol.* 54:401-407.
- Margaritte, *et al.* (1992). *Am. J. Hum. Genet.* 50:1231-1234.
- Margolskee (1992). *Curr. Top. Microbiol. Immunol.* 158:67-90.
- Matthews & Kricka (1988). *Anal. Biochem.* 169:1.
- Merrifield (1963). *J. Am. Chem. Soc.* 85:2149-2156.
- Metzger, *et al.* (1988). *Nature* 334:31-36.
- Miller (1992). *Curr. Top. Microbiol. Immunol.* 158:1-24.
- Miller, *et al.* (1985). *Mol. Cell. Biol.* 5:431-437.
- Miller, *et al.* (1988). *J. Virol.* 62:4337-4345.
- Mittlin (1989). *Clinical Chem.* 35:1819.
- Modrich, P. (1991). *Ann. Rev. Genet.* 25:229-253.
- Mombaerts, P., *et al.* (1992). *Cell* 68:869.
- Monaco, *et al.* 1986). *Nature* 323:646.
- Moss (1992). *Curr. Top. Microbiol. Immunol.* 158:25-38.
- Muzyczka (1992). *Curr. Top. Microbiol. Immunol.* 158:97-123.
- Nabel, *et al.* (1990). *Science* 249:1285-1288.
- Nabel (1992). *Hum. Gene Ther.* 3:399-410.
- Nakamura, *et al.* (1987). *Science* 235:1616-1622.
- Narod, S.A., *et al.* (1991) *The Lancet* 338:82-83.

- Newman, B., *et al.* (1988). *Proc. Natl. Acad. Sci. USA* **85**:3044-3048.
- Novack, *et al.* (1986). *Proc. Nat. Acad. Sci. USA* **83**:586.
- Oh, J. (1985). *Analysis of Human Genetic Linkage*, Johns Hopkins University Press, Baltimore, Md, pp. 1-216.
- Ohi, *et al.* (1990). *Gene* **89**:279-282.
- Oliphant, A., *et al.* (1991), *Nucleic Acid Res.* **19**:4794.
- Oliphant, A., *et al.* (1991). *Nucleic Acid Res.* **19**:4795.
- Orita, *et al.* (1989). *Proc. Nat. Acad. Sci. USA* **86**:276-2770.
- Orita, *et al.* (1989). *Genomics* **5**:874-879.
- Page, *et al.* (1990). *J. Virol.* **64**:5370-5276.
- Pellicer, *et al.* (1980). *Science* **209**:1414-1422.
- Petropoulos, *et al.* (1992). *J. Virol.* **66**:3391-3397.
- Philpott, K.L., *et al.* (1992). *Science* **256**:1448.
- Pierce, *et al.* (1992). *Proc. Natl. Acad. Sci. USA* **89**:2056-2060.
- Quantin, *et al.* (1992). *Proc. Natl. Acad. Sci. USA* **89**:2581-2584.
- Rano & Kidd (1989). *Nucl. Acids Res.* **17**:8392.
- Rosenfeld, *et al.* (1992). *Cell* **68**:143-155.
- Sambrook, J., *et al.* (1989). *Molecular cloning: A laboratory manual*, 2nd Ed. (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- Scharf, (1986). *Science* **233**:1076.
- Scopes, R, (1982). *Protein Purification: Principles and Practice*, (Springer-Verlag, N.Y.).
- Sheffield, V.C., *et al.* (1989). *Proc. Nat. Acad. Sci. USA* **86**: 232-236.
- Shenk, *et al.* (1975). *Proc. Nat. Acad. Sci. USA* **72**:989.
- Shimada, *et al.* (1991). *J. Clin. Invest.* **88**:1043-1047.
- Shinkai, Y., *et al.* (1992). *Cell* **68**:855.

- Skolnick, M.H. (1980). Banbury Report No 4: Cancer Incidence in Defined Populations (J. Cairns, J. Lyon, M. Skolnick, eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., pp. 285-297.
- Skolnick, M.H. and Wallace, B.R. (1988). *Genomics* 2:273-279.
- Skolnick, M.H. (1977). *Genet. Hum. Cancer*, (J.J. Mulvill, R.W. Miller and J.F. Freeman, Jr., eds.), Raven Press, N.Y.
- Skolnick, M.H., *et al.* (1990). *Science* 250:1715-1720.
- Smith, S.A., *et al.* (1992). *Nature Genetics* 2: 128-131.
- Snouwaert, J.N., *et al.* (1992). *Science* 257:1083.
- Sorge, *et al.* (1984). *Mol. Cell. Biol.* 4:1730-1737.
- Sternberg (1990). *Proc. Natl. Acad. Sci. USA* 87:103-107.
- Sternberg, *et al.* (1990). *The New Biologist* 2:151-162.
- Stewart, *et al.* (1992). *Hum. Gene Ther.* 3:267-275.
- Stratford-Perricaudet, *et al.* (1990). *Hum. Gene Ther.* 1:241-256.
- Tonolio, D., *et al.* (1990). Cold Spring Harbor Conference.
- Valancius, V. & Smithies, O. (1991). *Mol. Cell Biol.* 11:1402.
- van Dilla, *et al.* (1986). *Biotechnology* 4:537-552.
- Wagner, *et al.* (1991). *Proc. Natl. Acad. Sci. USA* 88:4255-4259.
- Wagner, *et al.* (1990). *Proc. Natl. Acad. Sci. USA* 87:3410-3414.
- Wang and Huang (1989). *Biochemistry* 28:9508-9514.
- Wartell, R.M., *et al.* (1990). *Nucl. Acids Res.* 18:2699-2705.
- Weber and May (1989). *Am. J. Hum. Genet.* 44:388-396.
- Weber, J.L. (1990). *Genomics* 7:524-530.
- Weber, J.L., *et al.* (1990). *Nucleic Acid Res.* 18:4640.
- Wells, J.A. (1991). *Methods Enzymol.* 202:390-411.
- Wetmur & Davidson (1968). *J. Mol. Biol.* 31:349-370.

- White & Lalouel (1988). *Ann. Rev. Genet.* **22**:259-279.
- Wilkinson, *et al.* (1992). *Nucleic Acids Res.* **20**:2233-2239.
- Willams and Anderson (1984). *Genet. Epidemiol.* **1**:7-20.
- Wolff, *et al.* (1990). *Science* **247**:1465-1468.
- Wolff, *et al.* (1991). *BioTechniques* **11**:474-485.
- Wu, *et al.* (1989). *J. Biol. Chem.* **264**:16985-16987.
- Wu, *et al.* (1991). *J. Biol. Chem.* **266**:14338-14342.
- Wu, *et al.* (1989). *Genomics* **4**:560-569.
- Zenke, *et al.* (1990). *Proc. Natl. Acad. Sci. USA* **87**:3655-3659.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Skolnick, Mark H.
Goldgar, David E.
Miki, Yoshio
Swenson, Jeff
Kamb, Alexander
Harshman, Keith D.
Shattuck-Eidens, Donna M.
Tavtigian, Sean V.
- (ii) TITLE OF INVENTION: 17q-Linked Breast and Ovarian Cancer
Susceptability Gene
- (iii) NUMBER OF SEQUENCES: 31
- (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Venable, Baetjer, Howard & Civiletti
 - (B) STREET: 1201 New York Avenue, N.W., Suite 1000
 - (C) CITY: Washington
 - (D) STATE: DC
 - (F) ZIP: 20005
- (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: Floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: PatentIn Release #1.0, Version #1.25
- (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER: US
 - (B) FILING DATE: 12-AUG-1994
 - (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
 - (A) APPLICATION NUMBER: US 08/246,602
 - (B) FILING DATE: 20-MAY-1994
- (viii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Ihnen, Jeffrey L.
 - (B) REGISTRATION NUMBER: 28,957
 - (C) REFERENCE/DOCKET NUMBER: 24884-109347-1
- (ix) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 202-962-4810
 - (B) TELEFAX: 202-962-8300

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 5220 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

GAAACCAAGTC TCAGTGTCCA ACTCTCTAAC CTTGGAACCTT GTGAGAACTC TGAGGACAAA	60
GCAGCGGATA CAACCTCAAA AGACGTCTGT CTACATTGAA TTGGCTGCTT GTGAATTTTC	120
TGAGACGGAT GTAACAAATA CTGAACATCA TCAACCCAGT AATAATGATT TGAACACCAC	180
TGAGAAGCGT GCAGCTGAGA GGCATCCAGA AAAGTATCAG GGTAGTTCTG TTTCAAACCTT	240
GCATGTGGAG CCATGTGGCA CAAATACTCA TGCCAGCTCA TTACAGCATG AGAACAGCAG	300
TTTATTACTC ACTAAAGACA GAATGAATGT AGAAAAGGCT GAATTCTGTA ATAAAAGCAA	360
ACAGCCTGGC TTAGCAAGGA GCCAACATAA CAGATGGGCT GGAAGTAAGG AAACATGTAA	420
TGATAGGCGG ACTCCCAGCA CAGAAAAAAA GGTAGATCTG AATGCTGATC CCCTGTGTGA	480
GAGAAAAGAA TGGAATAAGC AGAAACTGCC ATGCTCAGAG AATCCTAGAG ATACTGAAGA	540
TGTTCCCTTG ATAACACTAA ATAGCAGCAT TCAGAAAGTT AATGAGTGGT TTTCCAGAAG	600
TGATGAACTG TTAGGTTCTG ATGACTCACA TGATGGGGAG TCTGAATCAA ATGCCAAAGT	660
AGCTGATGTA TTGGACGTCC TAATTGAGGT AGATGAATAT TCTGGTTCTT CAGAGAAAAT	720
AGACTTACTG GCCAGTGATC CTCATGAGGC TTTAATATGT AAAAGTGACA GAGTTCACCTC	780
CAAATCAGTA GAGAGTAATA TTGAAGGCCA AATATTTGGG AAAACCTATC GGAAGAAGGC	840
AAGCCTCCCC AACTTAAGCC ATGTAAGTGA AAATCTAATT ATAGGGCATT TGTTCCTGAG	900
CCACAGATAA TACAAGAGCG TCCCCTCACA AATAAATTAA AGCGTAAAAG GAGACCTACA	960
TCAGGCCTTC ATCCTGAGGA TTTTATCAAG AAAGCAGATT TGGCAGTTCA AAAGACTCCT	1020
GAAATGATAA ATCAGGGAAC TAACCAAACG GAGCAGAATG GTCAAGTGAT GAATATTACT	1080
AATAGTGGTC ATGAGAATAA AACAAAAGGT GATTCTATTC AGAATGAGAA AAATCCTAAC	1140
CCAATAGAAT CACTCGAAAA AGAATCTGCT TTCAAAACGA AAGCTGAACC TATAAGCAGC	1200
AGTATAAGCA ATATGGAACCT CGAATTAAAT ATCCACAATT CAAAAGCACC TAAAAAGAAT	1260
AGGCTGAGGA GGAAGTCTTC TACCAGGCAT ATTCATGCGC TTGAACTAGT AGTCAGTAGA	1320
AATCTAAGCC CACCTAATTG TACTGAATTG CAAATTGATA GTTGTCTTAG CAGTGAAGAG	1380
ATAAAGAAAA AAAAGTACAA CCAAATGCCA GTCAGGCACA GCAGAAACCT ACAACTCATG	1440
GAAGGTAAAG AACCTGCAAC TGGAGCCAAG AAGAGTAACA AGCCAAATGA ACAGACAAGT	1500
AAAAGACATG ACAGTGATAC TTTCCCAGAG CTGAAGTTAA CAAATGCACC TGGTTCCTTTT	1560
ACTAAGTGTT CAAATACCAG TGAACCTAAA GAATTTGTCA ATCCTAGCCT TCCAAGAGAA	1620
GAAAAAGAAG AGAACTAGA AACAGTTAAA GTGTCTAATA ATGCTGAAGA CCCCAGAGAT	1680
CTCATGTTAA GTGGAGAAAG GGTTTTGCAA ACTGAAAGAT CTGTAGAGAG TAGCAGTATT	1740
TCACTGGTAC CTGGTACTGA TTATGGCACT CAGGAAAGTA TCTCGTTACT GGAAGTTAGC	1800
ACTCTAGGGA AGGCAAAAAC AGAACCAAAT AAATGTGTGA GTCAGTGTGC AGCATTTGAA	1860
AACCCCAAGG GACTAATTCA TGGTTGTTCC AAAGATAATA GAAATGACAC AGAAGGCTTT	1920

AAGTATCCAT	TGGGACATGA	AGTTAACCAC	AGTCGGGAAA	CAAGCATAGA	AATGGAAGAA	1980
AGTGAACTTG	ATGCTCAGTA	TTTGCAGAAT	ACATTCAAGG	TTTCAAAGCG	CCAGTCATTT	2040
GCTCTGTTTT	CAAATCCAGG	AAATGCAGAA	GAGGAATGTG	CAACATTCTC	TGCCCCACTCT	2100
GGGTCCTTAA	AGAAACAAAG	TCCAAAAGTC	ACTTTTGAAT	GTGAACAAAA	GGAAGAAAAT	2160
CAAGGAAAGA	ATGAGTCTAA	TATCAAGCCT	GTACAGACAG	TTAATATCAC	TGCAGGCTTT	2220
CCTGTGGTTG	GTCAGAAAGA	TAAGCCAGTT	GATAATGCCA	AATGTAGTAT	CAAAGGAGGC	2280
TCTAGGTTTT	GTCTATCATC	TCAGTTCAGA	GGCAACGAAA	CTGGACTCAT	TACTCCAAAT	2340
AAACATGGAC	TTTTACAAAA	CCCATATCGT	ATACCACCAC	TTTTTCCCAT	CAAGTCATTT	2400
GTTAAAACTA	AATGTAAGAA	AAATCTGCTA	GAGGAAAACT	TTGAGGAACA	TTCAATGTCA	2460
CCTGAAAGAG	AAATGGGAAA	TGAGAACATT	CCAAGTACAG	TGAGCACAAT	TAGCCGTAAT	2520
AACATTAGAG	AAAATGTTTT	TAAAGAAGCC	AGCTCAAGCA	ATATTAATGA	AGTAGGTTCC	2580
AGTACTAATG	AAGTGGGCTC	CAGTATTAAT	GAAATAGGTT	CCAGTGATGA	AAACATTCAA	2640
GCAGAACTAG	GTAGAAACAG	AGGGCCAAAA	TTGAATGCTA	TGCTTAGATT	AGGGGTTTTG	2700
CAACCTGAGG	TCTATAAACA	AAGTCTTCCT	GGAAGTAATT	GTAAGCATCC	TGAAATAAAA	2760
AAGCAAGAAT	ATGAAGAAGT	AGTTCAGACT	GTTAATACAG	ATTTCTCTCC	ATCTCTGATT	2820
TCAGATAACT	TAGAACAGCC	TATGGGAAGT	AGTCATGCAT	CTCAGGTTTG	TTCTGAGACA	2880
CCTGATGACC	TGTTAGATGA	TGGTGAAATA	AAGGAAGATA	CTAGTTTTGC	TGAAATGACA	2940
TTAAGGAAAG	TTCTGCTGTT	TTTAGCAAAA	GCGTCCAGAG	GAGAGCTTAG	CAGGGTCCTA	3000
GCCCTTTCAC	CCATACACAT	TTGGCTCAGG	GTTACCGAAG	AGGGGCCAAG	AAATTAGAGT	3060
CCTCAGAAGA	GAAC TTATCT	AGTGAGGATG	AAGAGCTTCC	CTGCTTCCAA	CACTTGTTAT	3120
TTGGTAAAGT	AAACAATATA	CCTTCTCAGT	CTACTAGGCA	TAGCACCGTT	GCTACCGAGT	3180
GTCTGTCTAA	GAACACAGAG	GAGAATTTAT	TATCATTGAA	GAATAGCTTA	AATGACTGCA	3240
GTAACCAGGT	AATATTGGCA	AAGGCATCTC	AGGAACATCA	CCTTAGTGAG	GAAACAAAAT	3300
GTTCTGCTAG	CTTGTTTTCT	TCACAGTGCA	GTGAATTGGA	AGACTTGACT	GCAAATACAA	3360
ACACCCAGGC	TCCTTTCTTG	ATTGGTTCTT	CCAAACAAAT	GAGGCATCAG	TCTGAAAGCC	3420
AGGGAGTTGG	TCTGAGTGAC	AAGGAATTGG	TTTCAGATGA	TGAAGAAAGA	GGAACGGGCT	3480
TGGAAGAAAA	TAATCAAGAA	GAGCAAAGCA	TGGATTCAAA	CTTAGGTGAA	GCAGCATCTG	3540
GGTGTGAGAG	TGAAACAAGC	GTCTCTGAAG	ACTGCTCAGG	GCTATCCTCT	CAGAGTGACA	3600
TTTTAACCAC	TCAGCAGAGG	GATACCATGC	AACATAACCT	GATAAAGCTC	CAGCAGGAAA	3660
TGGCTGAACT	AGAAGCTGTG	TTAGAACAGC	ATGGGAGCCA	GCCTTCTAAC	AGCTACCCTT	3720
CCATCATAAG	TGACTCTTCT	GCCCTTGAGG	ACCTGCGAAA	TCCAGAACAA	AGCACATCAG	3780
AAAAAGTATT	AACTTCACAG	AAAAGTAGTG	AATACCCTAT	AAGCCAGAAT	CCAGAAGGCC	3840
TTCTGCTGAC	AAGTTTGAGG	TGTCTGCAGA	TAGTTCTACC	AGTAAAAATA	AAGAACCAGG	3900
AGTGGAAGG	TCATCCCCCT	CTAAATGCCC	ATCATTAGAT	GATAGGTGGT	ACATGCACAG	3960

```

TTGCTCTGGG AGTCTTCAGA ATAGAACTA CCCACCTCAA GAGGAGCTCA TTAAGGTTGT      4020
TGATGTGGAG GAGCAACAGC TGGAAGAGTC TGGGCCACAC GATTTGACGG AAACATCTTA      4080
CTTGCCAAGG CAAGATCTAG AGGGAACCCC TTACCTGGAA TCTGGAATCA GCCTCTTCTC      4140
TGATGACCCT GAATCTGATC CTTCTGAAGA CAGAGCCCCA GAGTCAGCTC GTGTTGGCAA      4200
CATACCATCT TCAACCTCTG CATTGAAAGT TCCCCAATTG AAAGTTGCAG AATCTGCCCCA      4260
GAGTCCAGCT GCTGCTCATA CTACTGATAC TGCTGGGTAT AATGCAATGG AAGAAAGTGT      4320
GAGCAGGGAG AAGCCAGAAT TGACAGCTTC AACAGAAAGG GTCAACAAAA GAATGTCCAT      4380
GGTGGTGTCT GGCCTGACCC CAGAAGAATT TATGCTCGTG TACAAGTTTG CCAGAAAACA      4440
CCACATCACT TTAACCTAATC TAATTACTGA AGAGACTACT CATGTTGTTA TGAAAACAGA      4500
TGCTGAGTTT GTGTGTGAAC GGACACTGAA ATATTTTCTA GGAATTGCGG GAGGAAAATG      4560
GGTAGTTAGC TATTTCTGGG TGACCCAGTC TATTAAAGAA AGAAAAATGC TGAATGAGCA      4620
TGATTTTGAA GTCAGAGGAG ATGTGGTCAA TGGAAGAAAC CACCAAGGTC CAAAGCGAGC      4680
AAGAGAATCC CAGGACAGAA AGATCTTCAG GGGGCTAGAA ATCTGTTGCT ATGGGCCCTT      4740
CACCAACATG CCCACAGATC AACTGGAATG GATGGTACAG CTGTGTGGTG CTTCTGTGGT      4800
GAAGGAGCTT TCATCATTCA CCCTTGGCAC AGGTGTCCAC CCAATTGTGG TTGTGCAGCC      4860
AGATGCCTGG ACAGAGGACA ATGGCTTCCA TGCAATTGGG CAGATGTGTG AGGCGCCTGT      4920
GGTGACCCGA GAGTGGGTGT TGGACAGTGT AGCACTCTAC CAGTGCCAGG AGCTGGACAC      4980
CTACCTGATA CCCAGATCC CCCACAGCCA CTACTGACTG CAGCCAGCCA CAGGTACAGA      5040
GCCACAGGAC CCCAAGAATG AGCTTACAAA GTGGCCTTTC CAGGCCCTGG GAGCTCCTCT      5100
CACTCTTCAG TCCTTCTACT GTCCTGGCTA CTAAATATTT TATGTACATC AGCCTGAAAA      5160
GGACTTCTGG CTATGCAAGG GTCCCTTAAA GATTTTCTGC TTGAAGTCTC CCTTGGAAT      5220

```

(2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 1673 amino acids
 - (B) TYPE: amino acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: C-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Lys Pro Val Ser Val Ser Asn Ser Leu Thr Leu Glu Leu Val Arg Thr
1           5           10           15

```

Leu	Arg	Thr	Lys 20	Gln	Arg	Ile	Gln	Pro	Gln	Lys	Thr	Ser	Val 30	Tyr	Ile
Glu	Leu	Ala 35	Ala	Cys	Glu	Phe	Ser 40	Glu	Thr	Asp	Val	Thr 45	Asn	Thr	Glu
His	His 50	Gln	Pro	Ser	Asn	Asn 55	Asp	Leu	Asn	Thr	Thr 60	Glu	Lys	Arg	Ala
Ala 65	Glu	Arg	His	Pro	Glu 70	Lys	Tyr	Gln	Gly	Ser 75	Ser	Val	Ser	Asn	Leu 80
His	Val	Glu	Pro	Cys 85	Gly	Thr	Asn	Thr	His 90	Ala	Ser	Ser	Leu	Gln 95	His
Glu	Asn	Ser	Ser 100	Leu	Leu	Leu	Thr	Lys 105	Asp	Arg	Met	Asn	Val 110	Glu	Lys
Ala	Glu	Phe 115	Cys	Asn	Lys	Ser	Lys 120	Gln	Pro	Gly	Leu	Ala 125	Arg	Ser	Gln
His	Asn 130	Arg	Trp	Ala	Gly	Ser 135	Lys	Glu	Thr	Cys	Asn 140	Asp	Arg	Arg	Thr
Pro 145	Ser	Thr	Glu	Lys	Lys 150	Val	Asp	Leu	Asn	Ala 155	Asp	Pro	Leu	Cys	Glu 160
Arg	Lys	Glu	Trp	Asn 165	Lys	Gln	Lys	Leu	Pro 170	Cys	Ser	Glu	Asn	Pro 175	Arg
Asp	Thr	Glu	Asp 180	Val	Pro	Trp	Ile	Thr 185	Leu	Asn	Ser	Ser	Ile 190	Gln	Lys
Val	Asn	Glu 195	Trp	Phe	Ser	Arg	Ser 200	Asp	Glu	Leu	Leu	Gly 205	Ser	Asp	Asp
Ser	His 210	Asp	Gly	Glu	Ser	Glu 215	Ser	Asn	Ala	Lys	Val 220	Ala	Asp	Val	Leu
Asp 225	Val	Leu	Ile	Glu	Val 230	Asp	Glu	Tyr	Ser	Gly 235	Ser	Ser	Glu	Lys	Ile 240
Asp	Leu	Leu	Ala	Ser 245	Asp	Pro	His	Glu	Ala 250	Leu	Ile	Cys	Lys	Ser 255	Asp
Arg	Val	His 260	Ser	Lys	Ser	Val	Glu	Ser 265	Asn	Ile	Glu	Gly 270	Gln	Ile	Phe
Gly	Lys	Thr 275	Tyr	Arg	Lys	Lys	Ala 280	Ser	Leu	Pro	Asn	Leu 285	Ser	His	Val
Thr 290	Glu	Asn	Leu	Ile	Ile	Xaa 295	Ala	Phe	Val	Ser	Glu 300	Pro	Gln	Ile	Ile
Gln 305	Glu	Arg	Pro	Leu	Thr 310	Asn	Lys	Leu	Lys	Arg 315	Lys	Arg	Arg	Pro	Thr 320
Ser	Gly	Leu	His 325	Pro	Glu	Asp	Phe	Ile	Lys 330	Lys	Ala	Asp	Leu	Ala 335	Val
Gln	Lys	Thr 340	Pro	Glu	Met	Ile	Asn	Gln 345	Gly	Thr	Asn	Gln	Thr 350	Glu	Gln
Asn	Gly 355	Gln	Val	Met	Asn	Ile	Thr 360	Asn	Ser	Gly	His 365	Glu	Asn	Lys	Thr

Lys	Gly	Asp	Ser	Ile	Gln	Asn	Glu	Lys	Asn	Pro	Asn	Pro	Ile	Glu	Ser	370	375	380
Leu	Glu	Lys	Glu	Ser	Ala	Phe	Lys	Thr	Lys	Ala	Glu	Pro	Ile	Ser	Ser	385	390	395
Ser	Ile	Ser	Asn	Met	Glu	Leu	Glu	Leu	Asn	Ile	His	Asn	Ser	Lys	Ala	405	410	415
Pro	Lys	Lys	Asn	Arg	Leu	Arg	Arg	Lys	Ser	Ser	Thr	Arg	His	Ile	His	420	425	430
Ala	Leu	Glu	Leu	Val	Val	Ser	Arg	Asn	Leu	Ser	Pro	Pro	Asn	Cys	Thr	435	440	445
Glu	Leu	Gln	Ile	Asp	Ser	Cys	Ser	Ser	Ser	Glu	Glu	Ile	Lys	Lys	Lys	450	455	460
Lys	Tyr	Asn	Gln	Met	Pro	Val	Arg	His	Ser	Arg	Asn	Leu	Gln	Leu	Met	465	470	475
Glu	Gly	Lys	Glu	Pro	Ala	Thr	Gly	Ala	Lys	Lys	Ser	Asn	Lys	Pro	Asn	485	490	495
Glu	Gln	Thr	Ser	Lys	Arg	His	Asp	Ser	Asp	Thr	Phe	Pro	Glu	Leu	Lys	500	505	510
Leu	Thr	Asn	Ala	Pro	Gly	Ser	Phe	Thr	Lys	Cys	Ser	Asn	Thr	Ser	Glu	515	520	525
Leu	Lys	Glu	Phe	Val	Asn	Pro	Ser	Leu	Pro	Arg	Glu	Glu	Lys	Glu	Glu	530	535	540
Lys	Leu	Glu	Thr	Val	Lys	Val	Ser	Asn	Asn	Ala	Glu	Asp	Pro	Lys	Asp	545	550	555
Leu	Met	Leu	Ser	Gly	Glu	Arg	Val	Leu	Gln	Thr	Glu	Arg	Ser	Val	Glu	565	570	575
Ser	Ser	Ser	Ile	Ser	Leu	Val	Pro	Gly	Thr	Asp	Tyr	Gly	Thr	Gln	Glu	580	585	590
Ser	Ile	Ser	Leu	Leu	Glu	Val	Ser	Thr	Leu	Gly	Lys	Ala	Lys	Thr	Glu	595	600	605
Pro	Asn	Lys	Cys	Val	Ser	Gln	Cys	Ala	Ala	Phe	Glu	Asn	Pro	Lys	Gly	610	615	620
Leu	Ile	His	Gly	Cys	Ser	Lys	Asp	Asn	Arg	Asn	Asp	Thr	Glu	Gly	Phe	625	630	635
Lys	Tyr	Pro	Leu	Gly	His	Glu	Val	Asn	His	Ser	Arg	Glu	Thr	Ser	Ile	645	650	655
Glu	Met	Glu	Glu	Ser	Glu	Leu	Asp	Ala	Gln	Tyr	Leu	Gln	Asn	Thr	Phe	660	665	670
Lys	Val	Ser	Lys	Arg	Gln	Ser	Phe	Ala	Leu	Phe	Ser	Asn	Pro	Gly	Asn	675	680	685
Ala	Glu	Glu	Glu	Cys	Ala	Thr	Phe	Ser	Ala	His	Ser	Gly	Ser	Leu	Lys	690	695	700
Lys	Gln	Ser	Pro	Lys	Val	Thr	Phe	Glu	Cys	Glu	Gln	Lys	Glu	Glu	Asn	705	710	715

Gln	Gly	Lys	Asn	Glu	Ser	Asn	Ile	Lys	Pro	Val	Gln	Thr	Val	Asn	Ile	
				725					730					735		
Thr	Ala	Gly	Phe	Pro	Val	Val	Gly	Gln	Lys	Asp	Lys	Pro	Val	Asp	Asn	
			740					745					750			
Ala	Lys	Cys	Ser	Ile	Lys	Gly	Gly	Ser	Arg	Phe	Cys	Leu	Ser	Ser	Gln	
		755					760					765				
Phe	Arg	Gly	Asn	Glu	Thr	Gly	Leu	Ile	Thr	Pro	Asn	Lys	His	Gly	Leu	
	770					775					780					
Leu	Gln	Asn	Pro	Tyr	Arg	Ile	Pro	Pro	Leu	Phe	Pro	Ile	Lys	Ser	Phe	
785					790					795					800	
Val	Lys	Thr	Lys	Cys	Lys	Lys	Asn	Leu	Leu	Glu	Glu	Asn	Phe	Glu	Glu	
				805					810					815		
His	Ser	Met	Ser	Pro	Glu	Arg	Glu	Met	Gly	Asn	Glu	Asn	Ile	Pro	Ser	
			820					825					830			
Thr	Val	Ser	Thr	Ile	Ser	Arg	Asn	Asn	Ile	Arg	Glu	Asn	Val	Phe	Lys	
		835					840					845				
Glu	Ala	Ser	Ser	Ser	Asn	Ile	Asn	Glu	Val	Gly	Ser	Ser	Thr	Asn	Glu	
	850					855					860					
Val	Gly	Ser	Ser	Ile	Asn	Glu	Ile	Gly	Ser	Ser	Asp	Glu	Asn	Ile	Gln	
865					870					875					880	
Ala	Glu	Leu	Gly	Arg	Asn	Arg	Gly	Pro	Lys	Leu	Asn	Ala	Met	Leu	Arg	
				885					890					895		
Leu	Gly	Val	Leu	Gln	Pro	Glu	Val	Tyr	Lys	Gln	Ser	Leu	Pro	Gly	Ser	
		900						905					910			
Asn	Cys	Lys	His	Pro	Glu	Ile	Lys	Lys	Gln	Glu	Tyr	Glu	Glu	Val	Val	
		915					920					925				
Gln	Thr	Val	Asn	Thr	Asp	Phe	Ser	Pro	Ser	Leu	Ile	Ser	Asp	Asn	Leu	
	930					935					940					
Glu	Gln	Pro	Met	Gly	Ser	Ser	His	Ala	Ser	Gln	Val	Cys	Ser	Glu	Thr	
945					950					955					960	
Pro	Asp	Asp	Leu	Leu	Asp	Asp	Gly	Glu	Ile	Lys	Glu	Asp	Thr	Ser	Phe	
			965					970						975		
Ala	Xaa	Asn	Asp	Ile	Lys	Glu	Ser	Ser	Ala	Val	Phe	Ser	Lys	Ser	Val	
			980					985					990			
Gln	Xaa	Gly	Glu	Leu	Ser	Arg	Xaa	Pro	Ser	Pro	Phe	Thr	His	Thr	His	
		995					1000						1005			
Leu	Ala	Gln	Gly	Tyr	Arg	Arg	Gly	Ala	Lys	Lys	Leu	Glu	Ser	Ser	Glu	
	1010						1015				1020					
Glu	Asn	Leu	Ser	Ser	Glu	Asp	Glu	Glu	Leu	Pro	Cys	Phe	Gln	His	Leu	
	1025					1030				1035					1040	
Leu	Phe	Gly	Lys	Val	Asn	Asn	Ile	Pro	Ser	Gln	Ser	Thr	Arg	His	Ser	
				1045					1050					1055		
Thr	Val	Ala	Thr	Glu	Cys	Leu	Ser	Lys	Asn	Thr	Glu	Glu	Asn	Leu	Leu	
			1060					1065					1070			

Ser Leu Lys Asn Ser Leu Asn Asp Cys Ser Asn Gln Val Ile Leu Ala
1075 1080 1085

Lys Ala Ser Gln Glu His His Leu Ser Glu Glu Thr Lys Cys Ser Ala
1090 1095 1100

Ser Leu Phe Ser Ser Gln Cys Ser Glu Leu Glu Asp Leu Thr Ala Asn
1105 1110 1115 1120

Thr Asn Thr Gln Ala Pro Phe Leu Ile Gly Ser Ser Lys Gln Met Arg
1125 1130 1135

His Gln Ser Glu Ser Gln Gly Val Gly Leu Ser Asp Lys Glu Leu Val
1140 1145 1150

Ser Asp Asp Glu Glu Arg Gly Thr Gly Leu Glu Glu Asn Asn Gln Glu
1155 1160 1165

Glu Gln Ser Met Asp Ser Asn Leu Gly Glu Ala Ala Ser Gly Cys Glu
1170 1175 1180

Ser Glu Thr Ser Val Ser Glu Asp Cys Ser Gly Leu Ser Ser Gln Ser
1185 1190 1195 1200

Asp Ile Leu Thr Thr Gln Gln Arg Asp Thr Met Gln His Asn Leu Ile
1205 1210 1215

Lys Leu Gln Gln Glu Met Ala Glu Leu Glu Ala Val Leu Glu Gln His
1220 1225 1230

Gly Ser Gln Pro Ser Asn Ser Tyr Pro Ser Ile Ile Ser Asp Ser Ser
1235 1240 1245

Ala Leu Glu Asp Leu Arg Asn Pro Glu Gln Ser Thr Ser Glu Lys Val
1250 1255 1260

Leu Thr Ser Gln Lys Ser Ser Glu Tyr Pro Ile Ser Gln Asn Pro Glu
1265 1270 1275 1280

Gly Xaa Ser Ala Asp Lys Phe Glu Val Ser Ala Asp Ser Ser Thr Ser
1285 1290 1295

Lys Asn Lys Glu Pro Gly Val Glu Arg Ser Ser Pro Ser Lys Cys Pro
1300 1305 1310

Ser Leu Asp Asp Arg Trp Tyr Met His Ser Cys Ser Gly Ser Leu Gln
1315 1320 1325

Asn Arg Asn Tyr Pro Pro Gln Glu Glu Leu Ile Lys Val Val Asp Val
1330 1335 1340

Glu Glu Gln Gln Leu Glu Glu Ser Gly Pro His Asp Leu Thr Glu Thr
1345 1350 1355 1360

Ser Tyr Leu Pro Arg Gln Asp Leu Glu Gly Thr Pro Tyr Leu Glu Ser
1365 1370 1375

Gly Ile Ser Leu Phe Ser Asp Asp Pro Glu Ser Asp Pro Ser Glu Asp
1380 1385 1390

Arg Ala Pro Glu Ser Ala Arg Val Gly Asn Ile Pro Ser Ser Thr Ser
1395 1400 1405

Ala Leu Lys Val Pro Gln Leu Lys Val Ala Glu Ser Ala Gln Ser Pro
1410 1415 1420

Ala	Ala	Ala	His	Thr	Thr	Asp	Thr	Ala	Gly	Tyr	Asn	Ala	Met	Glu	Glu	1425		1430		1435		1440
Ser	Val	Ser	Arg	Glu	Lys	Pro	Glu	Leu	Thr	Ala	Ser	Thr	Glu	Arg	Val		1445		1450		1455	
Asn	Lys	Arg	Met	Ser	Met	Val	Val	Ser	Gly	Leu	Thr	Pro	Glu	Glu	Phe	1460		1465		1470		
Met	Leu	Val	Tyr	Lys	Phe	Ala	Arg	Lys	His	His	Ile	Thr	Leu	Thr	Asn	1475		1480		1485		
Leu	Ile	Thr	Glu	Glu	Thr	Thr	His	Val	Val	Met	Lys	Thr	Asp	Ala	Glu	1490		1495		1500		
Phe	Val	Cys	Glu	Arg	Thr	Leu	Lys	Tyr	Phe	Leu	Gly	Ile	Ala	Gly	Gly	1505		1510		1515		1520
Lys	Trp	Val	Val	Ser	Tyr	Phe	Trp	Val	Thr	Gln	Ser	Ile	Lys	Glu	Arg		1525		1530		1535	
Lys	Met	Leu	Asn	Glu	His	Asp	Phe	Glu	Val	Arg	Gly	Asp	Val	Val	Asn		1540		1545		1550	
Gly	Arg	Asn	His	Gln	Gly	Pro	Lys	Arg	Ala	Arg	Glu	Ser	Gln	Asp	Arg	1555		1560		1565		
Lys	Ile	Phe	Arg	Gly	Leu	Glu	Ile	Cys	Cys	Tyr	Gly	Pro	Phe	Thr	Asn	1570		1575		1580		
Met	Pro	Thr	Asp	Gln	Leu	Glu	Trp	Met	Val	Gln	Leu	Cys	Gly	Ala	Ser	1585		1590		1595		1600
Val	Val	Lys	Glu	Leu	Ser	Ser	Phe	Thr	Leu	Gly	Thr	Gly	Val	His	Pro		1605		1610		1615	
Ile	Val	Val	Val	Gln	Pro	Asp	Ala	Trp	Thr	Glu	Asp	Asn	Gly	Phe	His		1620		1625		1630	
Ala	Ile	Gly	Gln	Met	Cys	Glu	Ala	Pro	Val	Val	Thr	Arg	Glu	Trp	Val	1635		1640		1645		
Leu	Asp	Ser	Val	Ala	Leu	Tyr	Gln	Cys	Gln	Glu	Leu	Asp	Thr	Tyr	Leu	1650		1655		1660		
Ile	Pro	Gln	Ile	Pro	His	Ser	His	Tyr								1665		1670				

(2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: s754 A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:
CTAGCCTGGG CAACAAACGA

20

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: s754 B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:
GCAGGAAGCA GGAATGGAAC

20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:
(B) CLONE: s975 A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:
TAGGAGATGG ATTATTGGTG

20

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

- (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: s975 B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:
AGGCAACTTT GCAATGAGTG

20

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: tdj1474 A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:
CAGAGTGAGA CCTTGTCTCA AA

22

(2) INFORMATION FOR SEQ ID NO:8:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 23 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: tdj1474 B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TTCTGCAAAC ACCTTAAACT CAG

23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE: tdj1239 A

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AACCTGGAAG GCAGAGGTTG

20

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(vii) IMMEDIATE SOURCE:

(B) CLONE: tdj1239 B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

TCTGTACCTG CTAAGCAGTG G

21

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10952 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 341..443
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 920..4346
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 4347..4440
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 4750..4917
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 5136..5257
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 5921..6113
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 6700..6742
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 6910..7177
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 7691..7779
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 8582..8660
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 9482..9520
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 9966..10049
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 10316..10505
- (ix) FEATURE:
 - (A) NAME/KEY: exon
 - (B) LOCATION: 10625..10952

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CCCAAGNNNN AAACCCAATC ACAAANNCCN CNATTATAT CCNCCNCCAA TATTTGAAGA

60

GCCGGGTTCA	CCCCTTTCTA	ATCTGGTCTT	TGAACCNCT	GCCCCAAACA	ATCCTCCTCC	120
AYCCTCCCAA	RGTGCTGGGG	ATAATAGGCA	TGAACTNCCC	GCTCCAGCCC	AAGACAATTT	180
TAGTGTGAGC	AAAATTCCGG	GCATTTTTTC	AAGCHRDCAA	TACMTADCAT	GCWVRTGAHB	240
GGCTGATGWG	TCAATTATTT	TGCCCTGGTGC	AATTCTCTTC	AGGAGGAAAA	CACAMGACTG	300
CCAATAATTG	CTTGACTGTC	TTTACCAWAC	TGTTAARCAG	GAAACCARTC	TCAGTGTCCC	360
AACTCTCTAA	CCTTGGAAC	TGTGAGAACT	CTGAGGCAAA	GCAGCGGATA	CAACCTCAAA	420
AGACGTCTGT	CTACATTGAA	TTGGGTAAAG	TCTCAGGTTT	TTTAAGTATT	TAATAATAAT	480
TGCTGGATTC	CTTATCTTAT	AGTTTTGCCA	CAAATCTTGG	TCATAATTTG	TATTTGTGGT	540
AGGCAGCTTT	GGGAAGTGAA	TTTTATGAGC	CCTATGGTGA	GTTATAAAAA	ATGTAAAAGA	600
CGCAGTTCCN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	660
NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	720
ATCCTAAGTG	GAAATAATCT	AGGTAAATAG	GAATTAAATG	AAAGAGTATG	AGCTACATCT	780
TCAGTATACT	TGGTAGTTTA	TGAGGTTAGT	TTCTCTAATA	TAGCCAGTTG	GTTGATTTCC	840
ACCTCCAAGG	TGTATGAAGT	ATGTATTTTT	TTAATGACAA	TTCAGTTTGT	GAGTACCTTG	900
TTATTTNTGT	ATATTTTCAG	CTGCTTGTGA	ATTTTCTGAG	ACGGATGTAA	CAAATACTGA	960
ACATCATCAA	CCCAGTAATA	ATGATTTGAA	CACCACTGAG	AAGCGTGCAG	CTGAGAGGCA	1020
TCCAGAAAAG	TATCAGGGTA	GTTCTGTTTC	AACTTGCAT	GTGGAGCCAT	GTGGCACAAA	1080
TACTCATGCC	AGCTCATTAC	AGCATGAGAA	CAGCAGTTTA	TTACTCACTA	AAGACAGAAT	1140
GAATGTAGAA	AAGGCTGAAT	TCTGTAATAA	AAGCAAACAG	CCTGGCTTAG	CAAGGAGCCA	1200
ACATAACAGA	TGGGCTGGAA	GTAAGGAAAC	ATGTAATGAT	AGGCGGACTC	CCAGCACAGA	1260
AAAAAAGGTA	GATCTGAATG	CTGATCCCCT	GTGTGAGAGA	AAAGAATGGA	ATAAGCAGAA	1320
ACTGCCATGC	TCAGAGAATC	CTAGAGATAC	TGAAGATGTT	CCTTGGATAA	CACTAAATAG	1380
CAGCATTCAG	AAAGTTAATG	AGTGGTTTTT	CAGAAGTGAT	GAACTGTTAG	GTTCTGATGA	1440
CTCACATGAT	GGGGAGTCTG	AATCAAATGC	CAAAGTAGCT	GATGTATTGG	ACGTCTTAAT	1500
TGAGGTAGAT	GAATATTCTG	GTTCTTCAGA	GAAAATAGAC	TTACTGGCCA	GTGATCCTCA	1560
TGAGGCTTTA	ATATGTAAAA	GTGACAGAGT	TCACTCCAAA	TCAGTAGAGA	GTAATATTGA	1620
AGGCCAAATA	TTTGGGAAAA	CCTATCGGAA	GAAGGCAAGC	CTCCCCAACT	TAAGCCATGT	1680
AACTGAAAAT	CTAATTATAG	GNGCATTTGT	TTCTGAGCCA	CAGATAATAC	AAGAGCGTCC	1740
CCTCACAAAT	AAATTAAAGC	GTAAAAGGAG	ACCTACATCA	GGCCTTCATC	CTGAGGATTT	1800
TATCAAGAAA	GCAGATTTGG	CAGTTCAAAA	GACTCCTGAA	ATGATAAATC	AGGGAACTAA	1860
CCAAACGGAG	CAGAATGGTC	AAGTGATGAA	TATTACTAAT	AGTGGTCATG	AGAATAAAAC	1920
AAAAGGTGAT	TCTATTGAGA	ATGAGAAAAA	TCCTAACCCA	ATAGAATCAC	TCGAAAAAGA	1980
ATCTGCTTTC	AAAACGAAAG	CTGAACCTAT	AAGCAGCAGT	ATAAGCAATA	TGGAACTCGA	2040
ATTAAATATC	CACAATTCAA	AAGCACCTAA	AAAGAATAGG	CTGAGGAGGA	AGTCTTCTAC	2100

CAGGCATATT	CATGCGCTTG	AACTAGTAGT	CAGTAGAAAT	CTAAGCCCAC	CTAATTGTAC	2160
TGAATTGCAA	ATTGATAGTT	GTTCTAGCAG	TGAAGAGATA	AAGAAAAAAA	AGTACAACCA	2220
AATGCCAGTC	AGGCACAGCA	GAAACCTACA	ACTCATGGAA	GGTAAAGAAC	CTGCAACTGG	2280
AGCCAAGAAG	AGTAACAAGC	CAAATGAACA	GACAAGTAAA	AGACATGACA	GTGATACTTT	2340
CCCAGAGCTG	AAGTTAACAA	ATGCACCTGG	TTCTTTTACT	AAGTGTTCAA	ATACCAGTGA	2400
ACTTAAAGAA	TTTGTCAATC	CTAGCCTTCC	AAGAGAAGAA	AAAGAAGAGA	AACTAGAAAC	2460
AGTTAAAGTG	TCTAATAATG	CTGAAGACCC	CAAAGATCTC	ATGTTAAGTG	GAGAAAGGGT	2520
TTTGCAAAC	GAAAGATCTG	TAGAGAGTAG	CAGTATTTCA	CTGGTACCTG	GTACTGATTA	2580
TGGCACTCAG	GAAAGTATCT	CGTTACTGGA	AGTTAGCACT	CTAGGGAAGG	CAAAAACAGA	2640
ACCAAATAAA	TGTGTGAGTC	AGTGTGCAGC	ATTTGAAAAC	CCCAAGGGAC	TAATTCATGG	2700
TTGTTCCAAA	GATAATAGAA	ATGACACAGA	AGGCTTTAAG	TATCCATTGG	GACATGAAGT	2760
TAACCACAGT	CGGGAAACAA	GCATAGAAAT	GGAAGAAAGT	GAACTTGATG	CTCAGTATTT	2820
GCAGAATACA	TTCAAGGTTT	CAAAGCGCCA	GTCATTTGCT	CTGTTTTCAA	ATCCAGGAAA	2880
TGCAGAAGAG	GAATGTGCAA	CATTCTCTGC	CCACTCTGGG	TCCTTAAAGA	AACAAAGTCC	2940
AAAAGTCACT	TTTGAATGTG	AACAAAAGGA	AGAAAATCAA	GGAAAGAATG	AGTCTAATAT	3000
CAAGCCTGTA	CAGACAGTTA	ATATCACTGC	AGGCTTTCCT	GTGGTTGGTC	AGAAAGATAA	3060
GCCAGTTGAT	AATGCCAAAT	GTAGTATCAA	AGGAGGCTCT	AGGTTTTGTC	TATCATCTCA	3120
GTTTCAGAGGC	AACGAAACTG	GACTCATTAC	TCCAAATAAA	CATGGACTTT	TACAAAACCC	3180
ATATCGTATA	CCACCACTTT	TTCCCATCAA	GTCATTTGTT	AAAATAAAT	GTAAGAAAAA	3240
TCTGCTAGAG	GAAAACCTTG	AGGAACATTC	AATGTCACCT	GAAAGAGAAA	TGGGAAATGA	3300
GAACATTCCA	AGTACAGTGA	GCACAATTAG	CCGTAATAAC	ATTAGAGAAA	ATGTTTTTAA	3360
AGAAGCCAGC	TCAAGCAATA	TTAATGAAGT	AGGTTCCAGT	ACTAATGAAG	TGGGCTCCAG	3420
TATTAATGAA	ATAGGTTCCA	GTGATGAAAA	CATTCAAGCA	GAACTAGGTA	GAAACAGAGG	3480
GCCAAAATTG	AATGCTATGC	TTAGATTAGG	GGTTTTGCAA	CCTGAGGTCT	ATAAACAAAG	3540
TCTTCCTGGA	AGTAATTGTA	AGCATCCTGA	AATAAAAAAG	CAAGAATATG	AAGAAGTAGT	3600
TCAGACTGTT	AATACAGATT	TCTCTCCATC	TCTGATTTCA	GATAACTTAG	AACAGCCTAT	3660
GGGAAGTAGT	CATGCATCTC	AGGTTTGTTT	TGAGACACCT	GATGACCTGT	TAGATGATGG	3720
TGAAATAAAG	GAAGATACTA	GTTTTGCTGA	NAATGACATT	AAGGAAAGTT	CTGCTGTTTT	3780
TAGCAAAAGC	GTCCAGAGGG	GAGAGCTTAG	CAGGNGTCCT	AGCCCTTTCA	CCCATACACA	3840
TTTGGCTCAG	GGTTACCGAA	GAGGGGCCAA	GAAATTAGAG	TCCTCAGAAG	AGAACTTATC	3900
TAGTGAGGAT	GAAGAGCTTC	CCTGCTTCCA	ACACTTGTTA	TTTGGTAAAG	TAAACAATAT	3960
ACCTTCTCAG	TCTACTAGGC	ATAGCACCGT	TGCTACCGAG	TGTCTGTCTA	AGAACACAGA	4020
GGAGAATTTA	TTATCATTGA	AGAATAGCTT	AAATGACTGC	AGTAACCAGG	TAATATTGGC	4080
AAAGGCATCT	CAGGAACATC	ACCTTAGTGA	GGAAACAAAA	TGTTCTGCTA	GCTTGTTTTT	4140

TTCACAGTGC	AGTGAATTGG	AAGACTTGAC	TGCAAATACA	AACACCCAGG	CTCCTTTCTT	4200
GATTGGTTCT	TCCAAACAAA	TGAGGCATCA	GTCTGAAAGC	CAGGGAGTTG	GTCTGAGTGA	4260
CAAGGAATTG	GTTTCAGATG	ATGAAGAAAAG	AGGAACGGGC	TTGGAAGAAA	ATAATCAAGA	4320
AGAGCAAAGC	ATGGATTCAA	ACTTAGGTGA	AGCAGCATCT	GGGTGTGAGA	GTGAAACAAG	4380
CGTCTCTGAA	GACTGCTCAG	GGCTATCCTC	TCAGAGTGAC	ATTTTAACCA	CTCAGCAGAG	4440
VVVVVVVVVV	VVVGNGAAT	GTAATCCTAA	TATTTNCNC	CNACTTAAAA	GAATACCACT	4500
CCAANGGCAT	CNCAATACAT	CAATCAATTG	GGGAATTGGG	ATTTTCCCTC	NCTAACATCA	4560
NTGGAATAAT	TTCATGGCAT	TAATTGCATG	AATGTGGTTA	GATTAAAAGG	TGTTCATGCT	4620
AGAACTTGTA	GTTCCATACT	AGGTATTTCA	ATCNTTGTTT	CTCAAANAAT	TTGTATGATA	4680
TATTNTCATT	TAATGGAAAG	CTTCTCAAAG	TATTTCAATTT	TCTTGGTACC	ATMNATCGTT	4740
TTTGAATNAG	GGATACCATG	CAACATAACC	TGATAAAGCT	CCAGCAGGAA	ATGGCTGAAC	4800
TAGAAGCTGT	GTTAGAACAG	CATGGGAGCC	AGCCTTCTAA	CAGCTACCCT	TCCATCATAA	4860
GTGACTCTTC	TGCCCTTGAG	GACCTGCGAA	ATCCAGAACA	AAGCACATCA	GAAAAAGGTG	4920
TGTATTGTTG	GCCAAACACT	GATATCTTAA	NCAAAATTCT	TTCTTCCCC	TTTATCTCCT	4980
TCTGAAGAGT	AAGGACCTAG	CTCCAACATT	TTATGATCCT	TGCTCAGCAC	ATGGGTAATT	5040
ATGGAGCCTT	GGTTCTTGTC	CCTGCTCACA	ACTAATATAC	CAGTCAGAGG	GACCCAAGGC	5100
AGTCATTTCAT	GTTGTCATCT	GAGAVVVVVV	VVVVVTATTA	ACTTCACAGA	AAAGTAGTGA	5160
ATACCCTATA	AGCCAGAATC	CAGAAGGCCT	NTCTGCTGAC	AAGTTTGAGG	TGTCTGCAGA	5220
TAGTTCTACC	AGTAAAAATA	AAGAACCAGG	AGTGGAAVVV	VVVVGGATAA	GNTCAAGAGA	5280
TATTTTGATA	GGTGATGCAG	TGATNAATTG	NGAAAATTTN	CTGCCTGCTT	TTAATCTTCC	5340
CCCGTTCTTT	CTTCCCTNCCT	CCCTCCCTTC	CTNCCTCCCG	TCCTTNCCTT	TCCTTTCCCT	5400
CCCTTCCNCC	TTCTTTCCNT	CTNTCTTTCC	TTTCTTTCCCT	GTCTACCTTT	CTTTCCCTTCC	5460
TCCCTTCCCT	TTCTTTTCTT	TCTTTCCTTT	CCTTTTCTTT	CCTTTCCTTT	CTTTCCCTTTC	5520
TTTCTTGACA	GAGTCTTGCT	CTGTCACTCA	GGCTGGAGTG	CAGTGGCGTG	ATCTCGNCTC	5580
ACTGCAACCT	CTGTCTCCCA	GGTTCAAGCA	ATTTTCCTGC	CTCAGCCTCC	CGAGTAGCTG	5640
AGATTACAGG	CGCCAGCCAC	CACACCCAGC	TACTGACCTG	CTTTTVVVAA	ACAGCTGGGA	5700
GATATGGTGC	CTCAGACCAT	CCCCATGTTA	TATGTCAACC	CTGACATATT	GGCAGGCAAC	5760
ATGAATCCAG	ACTTCTAGGC	TGTCATGCGG	GCTCTTTCTT	TGCCAGTCAT	TNCTGATCTC	5820
TCTGACATGA	ACTGTNTCAG	GTATGCTTTG	GCTGCCCAGC	AAGTATGAAA	TGTCTTNTCA	5880
CAAGGGGTGG	CGATGGTTTT	CTCCTTCCAT	TTATCTTTCT	AGGTCATCCC	CTTCTAAATG	5940
CCCATCATTA	GATGATAGGT	GGTACATGCA	CAGTTGCTCT	GGGAGTCTTC	AGAAATAGAAA	6000
CTACCCACCT	CAAGAGGAGC	TCATTAAAGT	TGTTGATGTG	GAGGAGCAAC	AGCTGGAAGA	6060
GTCTGGGCCA	CACGATTTGA	CGGAAACATC	TTACTTGCCA	AGGCAAGATC	TAGGTAATAT	6120
TTCATCTGCT	GTATTGGARC	AMACACTYTG	ATTTTACTCT	GAATCCTACA	TAAAGATATT	6180

CTGGTTARCC	AACTTTTAGA	TGTRCTAGTC	TATCATGGAC	ACTTTTGTGA	TACTTAATTA	6240
AGCCCACTTT	AGAAAAATAG	CTCAAGTGTT	AATCWAGGTT	TACTTGWAAA	TTATTGAAAC	6300
TGTTAATCCA	TCTATATTTT	AATTAATGGT	TTAACTAATG	ATTTTGAGGA	TGWGGGAGTC	6360
KTGGTGTA	CTAMATGTAT	TATTTTCAGGC	CAGGCATAGT	GGCTCACGCC	TGGTAATCCC	6420
AGTAYYCMRG	AGCCCGAGGC	AGGTGGAGCN	NNNNNNNNNN	NNNNNNNNNN	NNNNTGTCTT	6480
GGCCAACATG	GGNGAAACCC	TGTCTTCTTC	TTAAAAANA	CAAAAAAAT	TAAGTGGGTT	6540
GTGCTTAGGT	GNATGCCCCG	NATCCTAGTT	NTTCTTGNGG	GTTGAGGGAG	GAGATCACNT	6600
TGGACCCCGG	AGGGGNGGGT	GGGGGNGAGC	AGGNCAAAAC	ACNGACCCAG	CTGGGGTGGA	6660
AGGGAAGCCC	ACTCNAAAAA	ANNTTNVVVV	VVVVVVVVVA	GGGAACCCCT	TACCTGGAAT	6720
CTGGAATCAG	CCTCTTCTCT	GAVVVVVVVV	VVVVVGTTGG	TTCCCCACN	TNANNTTGNC	6780
CATTTCCCCCT	ACNACTTNG	GGCTTTNAAC	NTAAAAANCC	AGNCNTTGAA	CCACATCCTC	6840
CTTNTNTAAA	TAAGTTCN	ATCCTTTAAG	GGGGNCCCT	TCCNGNAGN	GGNANCACCC	6900
YYTCYCYKRT	RTCCCTGAAT	CTGATCCTTC	TGAAGACAGA	GCCCCAGAGT	CAGCTCGTGT	6960
TGGCAACATA	CCATCTTCAA	CCTCTGCATT	GAAAGTTCCC	CAATTGAAAG	TTGCAGAATC	7020
TGCCCAGAGT	CCAGCTGCTG	CTCATACTAC	TGATACTGCT	GGGTATAATG	CAATGGAAGA	7080
AAGTGTGAGC	AGGGAGAAGC	CAGAATTGAC	AGCTTCAACA	GAAAGGGTCA	ACAAAAGAAT	7140
GTCCATGGTG	GTGTCTGGCC	TGACCCCAAG	AGAATTTGTG	AGTGTATCCA	TATGTATCTC	7200
CCTAATGACT	AAGACTTAAC	AACATTCTGG	AAAGAGTTTT	ATGTAGGTAT	TGTCAATTAA	7260
TAACCTAGAG	GAAGAAATCT	AGAAANCAAT	CACAGTTCTG	TGTAATTTAA	TTTCGATTAC	7320
TAATTTCTGA	AAATTTAGAA	YVVVVVVVVV	VVVVNCCCN	CCCCCNAATC	TGAAATGGGG	7380
GTAACCCCCC	CCCAACCGAN	ACNTGGGTNG	CNTAGAGANT	TTAATGGCCC	NTTCTGAGGN	7440
ACANAAGCTT	AAGCCAGGNG	ACGTGGANCN	ATGNGTTGTT	TNTTGTGTTG	TTACCTCCAG	7500
CCTGGTGACA	GANCAAGCTC	TGTCTNGNAA	AANTAGGNGA	GGGNNAAGAT	TCACNNTAAA	7560
TAGTTCCAGG	ACACGTGTAG	AACGTGCAGG	ATTGCTACGT	AGGTAAACAT	ATGCCATGGT	7620
GGGATAACTA	GTATTCTGAG	CTGTGTGCTA	GAGGTAAGTC	ATGATAATGG	AATATTTGAT	7680
TTAATTTTCA	ATGCTCGTGT	ACAAGTTTGC	CAGAAAACAC	CACATCACTT	TAAGTAACTT	7740
AATTACTGAA	GAGACTACTC	ATGTTGTTAT	GAAAACAGAT	ATACCCAAGA	ACCTTTACAG	7800
AATACCTTKC	ATCTSCTGCA	TAAAACCACA	TGAGGCGAGG	CACAGTGGCG	CATGCCTGTA	7860
WTCGCNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNTACCCCG	AGRTTMSGAG	TCGAGCCATN	7920
NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNAAATGGNN	NNNNNNNNNN	NNNNNNNNNN	7980
NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	8040
NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	8100
NNNNNNNNNN	NNNNNNNNNN	NNNNNNAACG	TGAACAAATA	AGAATATTTG	TTGAGCATAG	8160
CATGGATGAT	AGTCTTCTAA	TAGTCAATCA	ATTACTTTAT	GAAAGACAAA	TAATAGTTTT	8220

GCTGCTTCCT	TACCTCCTTT	TGTTTTGGGT	TAAGATTTGG	AGTGTGGGCC	AGGCACVVVV	8280
VVVVVVVVVG	ATCTATAGCT	AGCCTTGGCG	TCTAGAAGAT	GGGTGTTGAG	AAGAGGGAGT	8340
GGAAAGATAT	TTCCTCTGGT	CTTAACTTCA	TATCAGCCTC	CCCTAGACTT	CCAAATATCC	8400
ATACCTGCTG	GTTATAATTA	GTGGTGTTTT	CAGCCTCTGA	TTCTGTCACC	AGGGGTTTTA	8460
GAATCATAAA	TCCAGATTGA	TCTTGGGAGT	GTAAAAAACT	GAGGCTCTTT	AGCTTCTTAG	8520
GACAGCACTT	CCTGATTTTG	TTTTCAACTT	CTAATCCTTT	GAGTGKTCT	CATTCTGCAG	8580
ATGCTGAGTT	TGTGTGTGAA	CGGACACTGA	AATATTTTCT	AGGAATTGCG	GGAGGAAAAT	8640
GGGTAGTTAG	CTATTTCTGG	KAAGKATAAT	ACTATWTCTC	CCCYCCTCCC	CTAACAMCTC	8700
AGAATTGSAT	TTTACACCTA	ACATTTACAM	CTAAGNYYYT	KGCTGATGCT	TGAGTCTGAG	8760
TCMCMAAAGK	CCTTTAATTG	TAATACTAAC	TACTTTTATC	TTTAATATCA	CTTTGTTCAG	8820
ATAAGCTGGT	GATGCTGGGA	AAATGGTCTC	TTTTATAACT	AATAGGACCT	AATCTGCTCC	8880
TAGCAATGTT	AGCATATGAG	CTAGGGATTT	ATTTAATAGT	CGGCAGGAAT	CCATGTGCAR	8940
CAGNCAAACT	TATAATGTTT	AAATTAAACA	TCAACTCTGT	CTCCAGAAGG	AAACTGCTGC	9000
TACAAGCCTT	ATTAAAGGGC	TGTGGCTTTA	GAGGGAAGGA	CCTCTCCTCT	GTCATTCTTC	9060
CTGTGCTCTT	TTGTGAATCG	CTGACCTCTC	TATCTCCGTG	AAAAGAGCAC	GTTCTTCTGC	9120
TGTATGTAAC	CTGTCTTTTC	TATGATCTCT	VVVVVVVVVV	NAAAAACGGG	GNNGGGANTG	9180
GGCCTTAAAN	CCAAAGGGCN	AACTCCCCAA	CCATTNAAAA	ANTGACNGGG	GATTATTAAA	9240
ANCGGCGGGA	AACATTTTAC	NGCCCAACTA	ATATTGTTAA	ATTAAAACCA	CCACCNCTGC	9300
NCCAAGGAGG	GACCTTCTCC	NNCAAANCTT	ATTAAAGGGC	CTGNGCCTTA	AAGAGGGAAG	9360
GACCCTCTCC	TCTGTCAATC	TTCCTGTGCT	CTNTTTGTGA	ATCGCTGACC	TCTCTATCTC	9420
CGTGAAAAAG	AGCACGTTCT	TCTGCTGTAT	GTAACCTGTC	TTTTCTATGA	TCTCTTTAGG	9480
GGTGACCCAG	TCTATTAAAG	AAAGAAAAAT	GCTGAATGAG	GTAAGTACTT	GATGTTACAA	9540
ACTAACCAGA	GATATTCAAT	CAGTCATATA	GTTAAAAATG	TATTTGCTTC	CTTCCATCAA	9600
TGCACCACTT	TCCTTAACAA	TGCACAAATT	TTCCATGATA	ATGAGGATCA	TCAAGAATTA	9660
TGCAGGCCTG	CACTGTGGCT	CATACCTATA	ATCCCAGCGC	TTTGGGAGGC	TGAGGCGCTT	9720
GGATCVVVVV	VVVVVTGCAC	CCCAAGACGG	ATGGGNNCTC	CCCCNCCNCT	ATTTTGATTT	9780
AGAAANTGGG	TTCCCATGNG	TAAGCNGTGC	GACCCCTGCC	TCAATGACTN	CTCCTAATCT	9840
CCCAAATTCT	AGGATAGNGG	GTGANCCCTC	CNCTGNCTGA	TCCNTAAAAT	GGACGTGTCT	9900
GCTNCNCCAC	TCCATTGAAG	GAGCTTCTCT	TTCTCTTATC	CTGATGGGTG	TGNTGTTTCT	9960
TTCAGCATGA	TTTTGAAGTC	AGAGGAGATG	TGGTCAATGG	AAGAAACCAC	CAAGGTCCAA	10020
AGCGAGCAAG	AGAATCCCAG	GACAGAAAGG	TAAAGCTCCC	TCCCTCAAGT	TGACNNAAAT	10080
CTCACCCAC	CACTCTGTAT	TCCACTCCCC	TTTGCAGAGA	TGGGCCGCTT	CATTTTGTAA	10140
GACTTATTAC	ATACATACAC	AGTGCTAGAT	ACTTTTACAC	AGGTTCTTTT	TTCACTCTTC	10200
CATCCCAACC	ACATAAATAA	GTATTGTCTC	TACTTTATGA	ATGATAAAAC	TAAGAGATTT	10260

AGAGAGGCTG TGTAATTTGG ATTCCCGTCT CGGGTTCAGA TCVVVVVVVV VVVVATCTT	10320
CAGGGGGCTA GAAATCTGTT GCTATGGGCC CTTACCAAC ATGCCACAG ATCAACTGGA	10380
ATGGATGGTA CAGCTGTGTG GTGCTTCTGT GGTGAAGGAG CTTTCATCAT TCACCCTTGG	10440
CACAGGTGTC CACCCAATTG TGGTTGTGCA GCCAGATGCC TGGACAGAGG ACAATGGCTT	10500
CCATGVVVVV VVVVVVVVCA GTAATCCTNA GAACTCATAC GACCGGGCCC CTGGAGTCGN	10560
TGNTTNGAGC CTAGTCCNGG AGAATGAATT GACACTAATC TCTGCTTGTG TTCTCTGTCT	10620
CCAGCAATTG GGCAGATGTG TGAGGCGCCT GTGGTGACCC GAGAGTGGGT GTTGGACAGT	10680
GTAGCACTCT ACCAGTGCCA GGAGCTGGAC ACCTACCTGA TACCCAGAT CCCCCACAGC	10740
CACTACTGAC TGCAGCCAGC CACAGGTACA GAGCCACAGG ACCCCAAGAA TGAGCTTACA	10800
AAGTGGCCTT TCCAGGCCCT GGGAGCTCCT CTCACTCTTC AGTCCTTCTA CTGTCCTGGC	10860
TACTAAATAT TTTATGTACA TCAGCCTGAA AAGGACTTCT GGCTATGCAA GGTCCCTTA	10920
AAGATTTTCT GCTTGAAGTC TCCCTTGGAA AT	10952

(2) INFORMATION FOR SEQ ID NO:12:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

TGCTTGACTG TCTTACCAWA CTGTAARCAG	30
----------------------------------	----

(2) INFORMATION FOR SEQ ID NO:13:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GGTAAGGTCT CAGGTTTTTT AAGTATTGCT

30

(2) INFORMATION FOR SEQ ID NO:14:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GTGAGTACCT TGTTATTTNT GTATATTTCA

30

(2) INFORMATION FOR SEQ ID NO:15:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATTTCCTTGGT GCCATTTATC GTTTTTGAAG

30

(2) INFORMATION FOR SEQ ID NO:16:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GTATTGTTGG CCAAACACTG ATATCTTAAG

30

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

AGATTTGTTT TCTCATTCCT TTTAAAGCAG

30

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

GTAAGAAACA TCAATGTAAA GATGCTGTGG

30

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

ATGGTTTTCT CCTTTCATTT ATCTTTCTAG

30

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GTAATATTTTCT ATCTGCTGTA TTGGARCAMA

30

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TTCCCNAGNAG NGGNANCACC CYYTCYCYKR

30

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GTGTATCCAT ATGTATCTCC CTAATGACTA

30

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

ATGATAATGG AATATTTGAT TTAATTTTCA

30

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GTATACCAAG AACCTTTACA GAATACCTTG

30

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

TAATCCTTTG AGTGTKTCTC ATTCTGCAGA

30

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

KAAGKATAAT ACTATWTCTC CCCYCCTCCC

30

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

TAACCTGTCT TTTCTATGAT CTCTTTAGGG

30

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:
GTAAGTACTT GATGTTACAA ACTAACCAGA 30

(2) INFORMATION FOR SEQ ID NO:29:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:
TATCCTGATG GGTGTGTTT GTTTTTTCAG 30

(2) INFORMATION FOR SEQ ID NO:30:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:
GTAAAGCTCC CTCCCTCAAG TTGACAAAAA 30

(2) INFORMATION FOR SEQ ID NO:31:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 30 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

-106-

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Homo sapiens

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

CTAATCTCTG CTTGTGTTCT CTGTCTCCAG

30

WHAT IS CLAIMED IS:

1. An isolated DNA consisting essentially of DNA coding for a BRCA1 polypeptide, said DNA selected from the group consisting of cDNA and genomic DNA.
- 5 2. The isolated DNA of claim 1, wherein said BRCA1 polypeptide comprises the amino acid sequence set forth in SEQ ID No:2.
3. An isolated DNA consisting essentially of DNA having at least 15 nucleotides of the DNA of claim 1.
4. The isolated DNA of claim 3 which comprises regulatory sequences.
- 10 5. A pair of single-stranded DNA primers for determination of a nucleotide sequence of an BRCA1 gene by a polymerase chain reaction, the sequence of said primers being derived from human chromosome 17q, wherein the use of said primers in a polymerase chain reaction results in the synthesis of DNA having all or part of the sequence of the BRCA1 gene.
- 15 6. The pair of primers of claim 5 wherein said sequence of the BRCA1 gene is set forth in SEQ ID No:1.
7. An isolated DNA selected from the group consisting of:
 - (a) SEQ ID NO:1 having T at nucleotide position 3330;
 - (b) SEQ ID NO:1 having extra C at nucleotide position 4656;
 - (c) SEQ ID NO:1 having G at nucleotide position 4714; and
 - 20 (d) SEQ ID NO:1 having A at nucleotide position 4513..
8. A nucleic acid probe complementary to human wild-type BRCA1 gene sequences.

9. A nucleic acid probe complementary to human altered BRCA1 gene sequences which hybridizes to part of the BRCA1 gene, said part of the BRCA1 gene selected from the group consisting of:
 - (a) SEQ ID NO:1 having T at nucleotide position 3330;
 - 5 (b) SEQ ID NO:1 having extra C at nucleotide position 4656;
 - (c) SEQ ID NO:1 having G at nucleotide position 4714; and
 - (d) SEQ ID NO:1 having A at nucleotide position 4513.
- 10 10. A replicative cloning vector which comprises the isolated DNA of claim 1 and a replicon operative in a host cell.
- 10 11. An expression system which comprises the isolated DNA of claim 1 operably linked to suitable control sequences.
12. Recombinant host cells transformed with the expression system of claim 11.
13. A method of producing recombinant BRCA1 polypeptide which comprises culturing the cells of claim 12 under conditions effective for the production of said BRCA1 polypeptide.
- 15 14. A preparation of human BRCA1 polypeptide substantially free of other human proteins, the amino acid sequence of said protein corresponding to that shown in SEQ ID NO:2.
- 15 15. A preparation of human BRCA1 polypeptide substantially free of other human proteins, the amino acid sequence of said polypeptide having substantial homology with the amino acid sequence set forth in SEQ ID NO:1, said polypeptide having substantially similar function as wild-type BRCA1 polypeptide.
- 20 16. An antibody immunoreactive with a human RCA1 polypeptide and not substantially immunoreactive with other human polypeptides.
17. The antibody of claim 16 which is a monoclonal antibody.

18. A method for diagnosing a polymorphism associated with predisposition for breast and ovarian cancer in a human comprising: detecting a germline alteration of the wild-type BRCA1 gene or its expression products in a human sample, said alteration indicating a predisposition to said cancer.
- 5 19. The method of claim 18 wherein said BRCA1 gene has the sequence set forth in SEQ ID NO:1.
20. The method of claim 18 wherein said expression product is mRNA of BRCA1.
21. The method of claim 20 wherein the alteration of the wild-type BRCA1 gene mRNA is detected by hybridization of the mRNA from said tissue sample to an BRCA1 gene probe.
- 10 22. The method of claim 18 wherein an alteration is detected in the regulatory regions of the BRCA1 gene.
23. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by observing shifts in electrophoretic mobility of single-stranded DNA on non-denaturing polyacrylamide gels.
- 15 24. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by hybridization of an BRCA1 gene probe to genomic DNA isolated from said tissue.
25. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by hybridization with an allele-specific probe.
- 20 26. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by amplifying all or part of the BRCA1 gene in said tissue to produce an amplified sequence and sequencing the amplified sequence.
27. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by amplifying all or part of the BRCA1 gene for a specific BRCA1 allele.

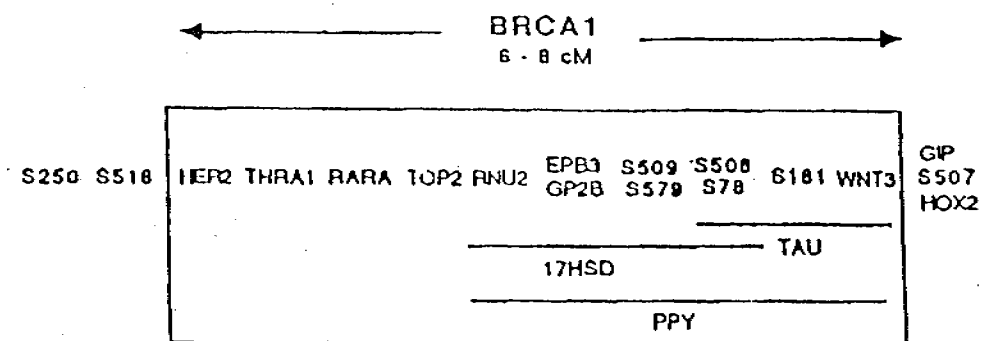
28. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by molecularly cloning all or part of the BRCA1 gene in said tissue to produce a cloned sequence and sequencing the cloned sequence.
- 5 29. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by identifying a mismatch between molecules (1) BRCA1 gene genomic DNA or BRCA1 mRNA isolated from said tissue, and (2) a nucleic acid probe complementary to the human wild-type BRCA1 gene DNA, when molecules (1) and (2) are hybridized to each other to form a duplex.
- 10 30. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by amplification of BRCA1 gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise wild-type BRCA1 gene sequences.
31. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by amplification of BRCA1 gene sequences in said tissue and hybridization of the amplified sequences to nucleic acid probes which comprise non-wild-type BRCA1 gene sequences.
- 15 32. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by screening for a deletion mutation.
33. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by screening for a point mutation.
- 20 34. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by screening for an insertion.
35. The method of claim 18 wherein the alteration of the wild-type BRCA1 gene is detected by *in situ* hybridization of the BRCA1 gene with nucleic acid probes which comprise the BRCA1 gene.
36. The method of claim 18 wherein the expression products are protein molecules.

37. The method of claim 36 wherein the alteration of wild-type BRCA1 gene protein is detected by immunoblotting.
38. The method of claim 36 wherein the alteration of wild-type BRCA1 gene protein is detected by immunocytochemistry.
- 5 39. The method of claim 36 wherein the alteration of wild-type BRCA1 gene protein is detected by assaying for binding interactions between BRCA1 gene protein isolated from said tissue and its ligand.
40. The method of claim 36 wherein the alteration of wild-type BRCA1 gene protein is detected by assaying for the inhibition of biochemical activity of a its binding partner.
- 10 41. A method for confirming the lack of a predisposition for cancer at the BRCA1 locus in a human, comprising: detecting the wild-type BRCA1 gene or its expression products in a human sample, the presence of the wild-type gene or expression products indicating no predisposition to cancer at the BRCA1 locus.
- 15 42. The method of claim 41 wherein said BRCA1 gene has the sequence set forth in SEQ ID NO:1.
43. The method of claim 41 wherein said expression product is mRNA of BRCA1.
44. The method of claim 41 wherein the expression products are protein molecules.
- 20 45. A method for diagnosing or prognosing tissue of a lesion in a human, comprising: detecting a somatic alteration of wild-type BRCA1 gene or its expression products in tissue isolated from said human, said alteration indicating mutation of said tissue.
46. The method of claim 45 wherein said BRCA1 gene has the sequence set forth in SEQ ID NO:1.

47. The method of claim 45 wherein said expression product is mRNA of BRCA1.
48. The method of claim 47 wherein the alteration of the wild-type BRCA1 gene mRNA is detected by hybridization of the mRNA from said tissue sample to an BRCA1 gene probe.
- 5 49. The method of claim 45 wherein the alteration is detected in the regulatory region of the BRCA1 gene.
50. The method of claim 45 wherein the expression products are protein molecules.
51. A method for confirming the lack of a mutation at the BRCA1 locus in a human, comprising: detecting the wild-type BRCA1 gene or its expression products in a human sample, the presence of the wild-type gene or expression products indicating no mutation
10 resulting from the BRCA1 locus.
52. The method of claim 51 wherein said BRCA1 gene has the sequence set forth in SEQ ID NO:1.
53. The method of claim 51 wherein said expression product is mRNA of BRCA1.
54. The method of claim 51 wherein the expression products are protein molecules.
- 15 55. A method for supplying a wild-type BRCA1 gene function or an BRCA1 function substantially similar to the wild-type to a cell which has lost said gene function or has altered gene function by virtue of a mutation in the BRCA1 gene, comprising: introducing a wild-type BRCA1 gene nucleic acid or nucleic acid substantially homologous to said wild-type BRCA1 gene nucleic acid and capable of suppression of a transformed state of said
20 cell.
56. The method of claim 55 wherein said nucleic acid is a portion of wild-type BRCA1 gene, said portion encoding a part of the wild-type BRCA1 gene polypeptide which is required for suppression of a transformed state of said cell.

57. The method of claim 55 wherein said nucleic acid is a portion of wild-type BRCA1 gene, said portion encoding all of the wild-type BRCA1 gene polypeptide which is required for suppression of a transformed state of said cell.
- 5 58. The method of claim 55 wherein said nucleic acid is substantially homologous to a portion of the wild-type BRCA1 gene, said portion encoding a polypeptide which is substantially homologous to part of the wild-type BRCA1 polypeptide and has a substantially similar function to the part of the wild-type BRCA1 polypeptide required for suppression of a transformed state of said cell.
- 10 59. The method of claim 55 wherein said nucleic acid is substantially homologous to a portion of the wild-type BRCA1 gene, said portion encoding a polypeptide which is substantially homologous to all of the wild-type BRCA1 polypeptide and has a substantially similar function to all of the wild-type BRCA1 polypeptide required for suppression of a transformed state of said cell.
- 15 60. The method of claim 55 wherein said nucleic acid contains the BRCA1 gene regulatory sequences.
61. The method of claim 55 wherein said nucleic acid is incorporated into the genome of said cell.
- 20 62. A method for supplying a wild-type BRCA1 gene function or an BRCA1 function substantially similar to the wild-type to a cell which has lost said gene function or has altered gene function by virtue of a mutation in the BRCA1 gene, comprising: introducing into the cell all or a part of a wild-type BRCA1 polypeptide which is required for suppression of a transformed state of said cell.
- 25 63. A method for supplying a wild-type BRCA1 gene function or an BRCA1 function substantially similar to the wild-type to a cell which has lost said gene function or has altered gene function by virtue of a mutation in the BRCA1 gene, comprising: introducing into the cell a molecule which mimics the function of wild-type BRCA1 polypeptide.

64. The method of claim 63 wherein said molecule is a polypeptide substantially homologous to the complete wild-type BRCA1 polypeptide required for suppression of a transformed state of said cell and has a substantially similar function as the wild-type BRCA1 polypeptide.
- 5 65. The method of claim 63 wherein said molecule is a polypeptide substantially homologous to a portion of the wild-type BRCA1 polypeptide required for suppression of a transformed state of said cell and has a substantially similar function as the portion of the wild-type BRCA1 polypeptide.
- 10 66. A method for screening potential cancer therapeutics which comprises: combining a BRCA1 binding partner, BRCA1 polypeptide and a compound suspected of being a cancer therapeutic and determining the amount of binding of the BRCA1 polypeptide to its binding partner.
67. A method for screening potential cancer therapeutics which comprises: combining a BRCA1 binding partner and a compound suspected of being a cancer therapeutic and measuring the biological activity of the binding partner.
- 15 68. A method for screening potential cancer therapeutics which comprises: growing a transformed eukaryotic host cell containing an altered BRCA1 gene in the presence of a compound suspected of being a cancer therapeutic and determining the rate of growth of said host cell.
- 20 69. A transgenic animal which carries an altered BRCA1 allele from a second animal in its genome.
70. The transgenic animal of claim 69 wherein the altered BRCA1 allele is a deletion.
71. The transgenic animal of claim 69 wherein the altered BRCA1 allele is a disrupted allele.



Map of the early onset breast and ovarian cancer region (BRCA1)

FIGURE 1

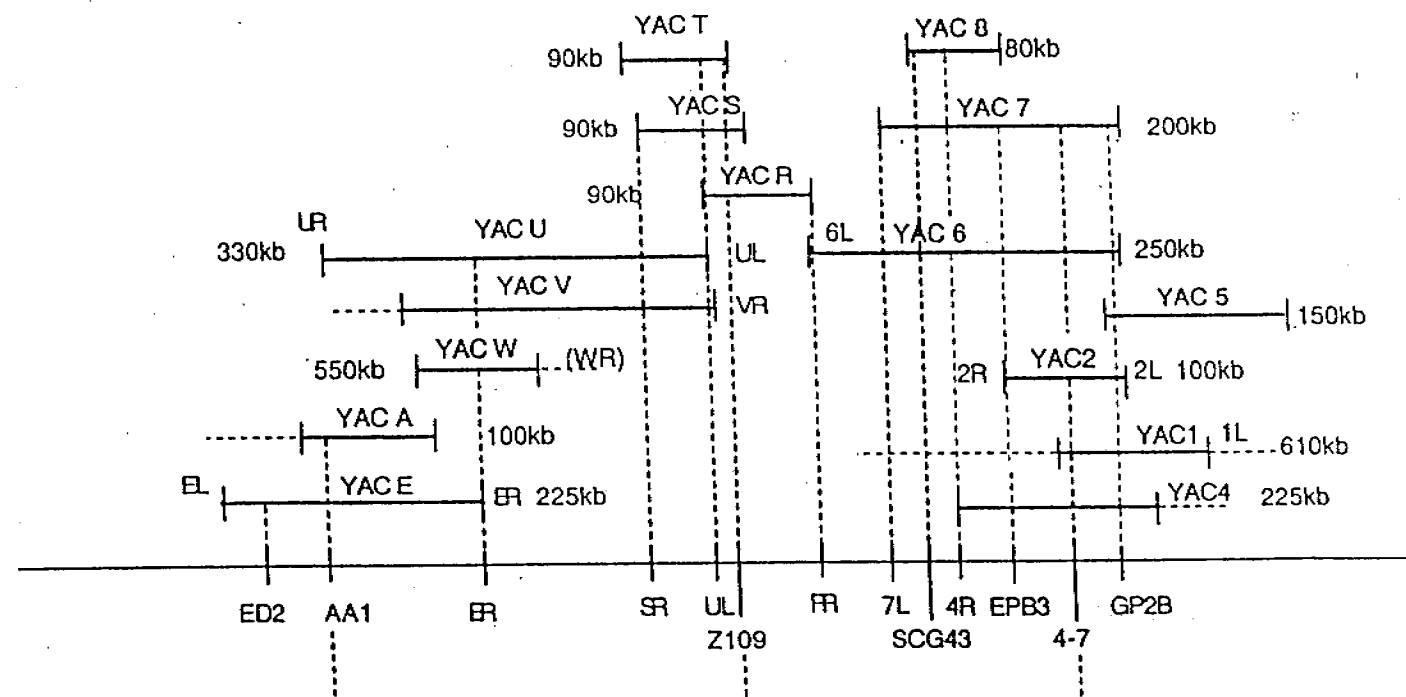


FIGURE 2

08/289221

Schematic Map of STS', Pls, and Bac's in the Region

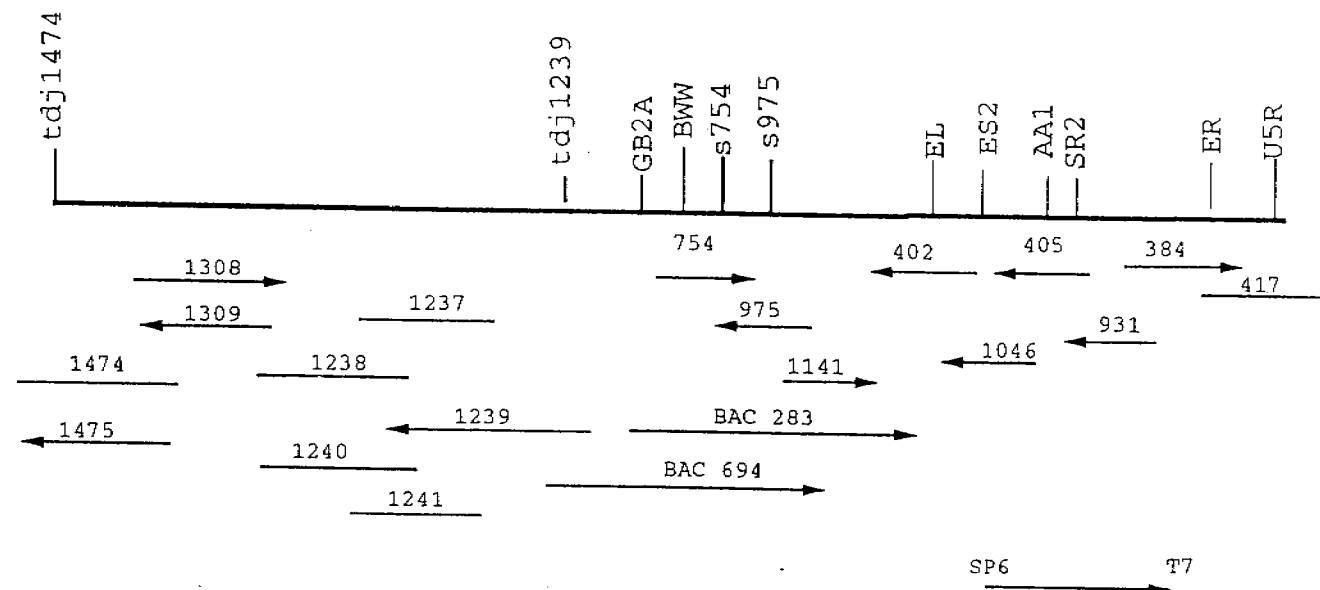


FIGURE 3

08/289221

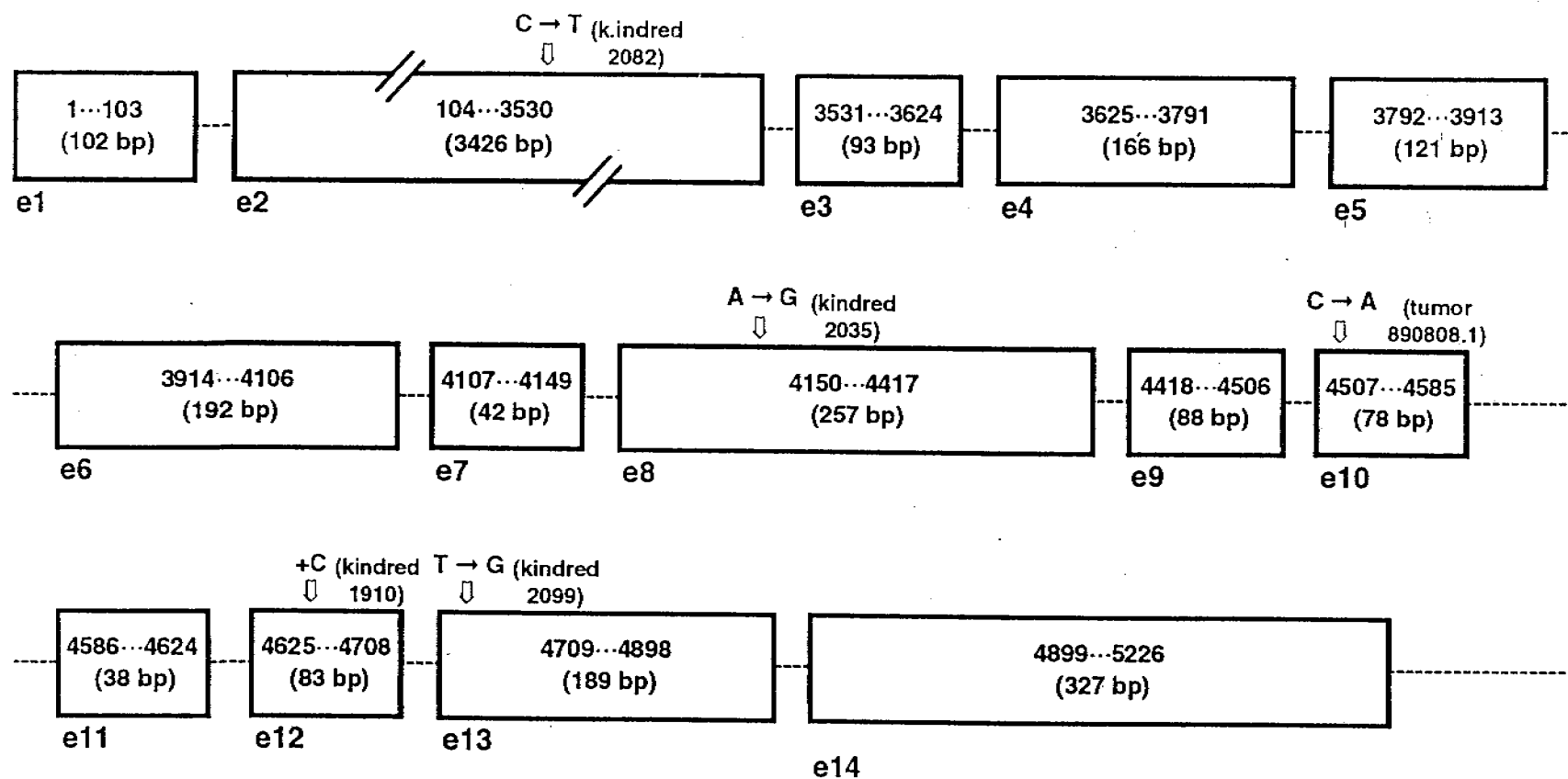


FIGURE 4

08/289221

CL

#2

PAGE: 1

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/289,221

DATE: 08/19/94
TIME: 18:05:56

INPUT SET: S8412.raw

This Raw Listing contains the General
Information Section and up to the first 5 pages.

SEQUENCE LISTING

ENTERED

(1) General Information:

(i) APPLICANT: Skolnick, Mark H.
Goldgar, David E.
Miki, Yoshio
Swenson, Jeff
Kamb, Alexander
Harshman, Keith D.
Shattuck-Eidens, Donna M.
Tavtigian, Sean V.

(ii) TITLE OF INVENTION: 17q-Linked Breast and Ovarian Cancer
Susceptability Gene

(iii) NUMBER OF SEQUENCES: 31

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Venable, Baetjer, Howard & Civiletti
(B) STREET: 1201 New York Avenue, N.W., Suite 1000
(C) CITY: Washington
(D) STATE: DC
(F) ZIP: 20005

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: US
(B) FILING DATE: 12-AUG-1994
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER: US 08/246,602
(B) FILING DATE: 20-MAY-1994

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Ihnen, Jeffrey L.
(B) REGISTRATION NUMBER: 28,957
(C) REFERENCE/DOCKET NUMBER: 24884-109347-1

(ix) TELECOMMUNICATION INFORMATION:

RAW SEQUENCE LISTING
PATENT APPLICATION US/08/289,221

DATE: 08/19/94
TIME: 18:06:01

INPUT SET: S8412.raw

47 (A) TELEPHONE: 202-962-4810
48 (B) TELEFAX: 202-962-8300
49
50
51 (2) INFORMATION FOR SEQ ID NO:1:
52
53 (i) SEQUENCE CHARACTERISTICS:
54 (A) LENGTH: 5220 base pairs
55 (B) TYPE: nucleic acid
56 (C) STRANDEDNESS: single
57 (D) TOPOLOGY: linear
58
59 (ii) MOLECULE TYPE: cDNA
60
61 (iii) HYPOTHETICAL: NO
62
63 (iv) ANTI-SENSE: NO
64
65 (vi) ORIGINAL SOURCE:
66 (A) ORGANISM: Homo sapiens
67
68
69 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
70
71 GAAACCAGTC TCAGTGTCCA ACTCTCTAAC CTTGGAACTT GTGAGAACTC TGAGGACAAA 60
72
73 GCAGCGGATA CAACCTCAAA AGACGTCTGT CTACATTGAA TTGGCTGCTT GTGAATTTTC 120
74
75 TGAGACGGAT GTAACAAATA CTGAACATCA TCAACCCAGT AATAATGATT TGAACACCAC 180
76
77 TGAGAAGCGT GCAGCTGAGA GGCATCCAGA AAAGTATCAG GGTAGTTCTG TTTCAAACCTT 240
78
79 GCATGTGGAG CCATGTGGCA CAAATACTCA TGCCAGCTCA TTACAGCATG AGAACAGCAG 300
80
81 TTTATTACTC ACTAAAGACA GAATGAATGT AGAAAAGGCT GAATTCTGTA ATAAAAGCAA 360
82
83 ACAGCCTGGC TTAGCAAGGA GCCAACATAA CAGATGGGCT GGAAGTAAGG AAACATGTAA 420
84
85 TGATAGGCGG ACTCCCAGCA CAGAAAAAAA GGTAGATCTG AATGCTGATC CCCTGTGTGA 480
86
87 GAGAAAAGAA TGGAATAAGC AGAAACTGCC ATGCTCAGAG AATCCTAGAG ATACTGAAGA 540
88
89 TGTTCCTTGG ATAACACTAA ATAGCAGCAT TCAGAAAGTT AATGAGTGGT TTTCCAGAAG 600
90
91 TGATGAACTG TTAGGTTCTG ATGACTCACA TGATGGGGAG TCTGAATCAA ATGCCAAAGT 660
92
93 AGCTGATGTA TTGGACGTCC TAATTGAGGT AGATGAATAT TCTGGTTCTT CAGAGAAAAAT 720
94
95 AGACTTACTG GCCAGTGATC CTCATGAGGC TTTAATATGT AAAAGTGACA GAGTTCACCTC 780
96
97 CAAATCAGTA GAGAGTAATA TTGAAGGCCA AATATTTGGG AAAACCTATC GGAAGAAGGC 840
98
99 AAGCCTCCCC AACTTAAGCC ATGTAAGTGA AAATCTAATT ATAGGGCATT TGTTTCTGAG 900

RAW SEQUENCE LISTING PATENT APPLICATION US/08/289,221

DATE: 08/19/94
TIME: 18:06:06

INPUT SET: S8412.raw

100									
101	CCACAGATAA	TACAAGAGCG	TCCCCTCACA	AATAAATTAA	AGCGTAAAAG	GAGACCTACA		960	
102									
103	TCAGGCCTTC	ATCCTGAGGA	TTTTATCAAG	AAAGCAGATT	TGGCAGTTCA	AAAGACTCCT		1020	
104									
105	GAAATGATAA	ATCAGGGAAC	TAACCAAACG	GAGCAGAATG	GTCAAGTGAT	GAATATTACT		1080	
106									
107	AATAGTGGTC	ATGAGAATAA	AACAAAAGGT	GATTCTATTG	AGAATGAGAA	AAATCCTAAC		1140	
108									
109	CCAATAGAAT	CACTCGAAAA	AGAATCTGCT	TTCAAAACGA	AAGCTGAACC	TATAAGCAGC		1200	
110									
111	AGTATAAGCA	ATATGGAACT	CGAATTAAAT	ATCCACAATT	CAAAAGCACC	TAAAAAGAAT		1260	
112									
113	AGGCTGAGGA	GGAAGTCTTC	TACCAGGCAT	ATTCATGCGC	TTGAACTAGT	AGTCAGTAGA		1320	
114									
115	AATCTAAGCC	CACCTAATTG	TACTGAATTG	CAAATTGATA	GTTGTTCTAG	CAGTGAAGAG		1380	
116									
117	ATAAAGAAAA	AAAAGTACAA	CCAAATGCCA	GTCAGGCACA	GCAGAAACCT	ACAACTCATG		1440	
118									
119	GAAGGTAAAG	AACCTGCAAC	TGGAGCCAAG	AAGAGTAACA	AGCCAAATGA	ACAGACAAGT		1500	
120									
121	AAAAGACATG	ACAGTGATAC	TTTCCCAGAG	CTGAAGTTAA	CAAATGCACC	TGGTTCTTTT		1560	
122									
123	ACTAAGTGTT	CAAATACCAG	TGAACTTAAA	GAATTTGTCA	ATCCTAGCCT	TCCAAGAGAA		1620	
124									
125	GAAAAAGAAG	AGAACTAGA	AACAGTTAAA	GTGTCTAATA	ATGCTGAAGA	CCCCAAAGAT		1680	
126									
127	CTCATGTTAA	GTGGAGAAAAG	GGTTTTGCAA	ACTGAAAGAT	CTGTAGAGAG	TAGCAGTATT		1740	
128									
129	TCACTGGTAC	CTGGTACTGA	TTATGGCACT	CAGGAAAGTA	TCTCGTTACT	GGAAGTTAGC		1800	
130									
131	ACTCTAGGGA	AGGCCAAAAC	AGAACCAAAT	AAATGTGTGA	GTCAGTGTGC	AGCATTTGAA		1860	
132									
133	AACCCCAAGG	GACTAATTCA	TGGTTGTTCC	AAAGATAATA	GAAATGACAC	AGAAGGCTTT		1920	
134									
135	AAGTATCCAT	TGGGACATGA	AGTTAACCAC	AGTCGGGAAA	CAAGCATAGA	AATGGAAGAA		1980	
136									
137	AGTGAACCTG	ATGCTCAGTA	TTTGCAGAAT	ACATTCAAGG	TTTCAAAGCG	CCAGTCATTT		2040	
138									
139	GCTCTGTTTT	CAAATCCAGG	AAATGCAGAA	GAGGAATGTG	CAACATTCTC	TGCCCCACTCT		2100	
140									
141	GGGTCCTTAA	AGAAACAAAG	TCCAAAAGTC	ACTTTTGAAT	GTGAACAAAA	GGAAGAAAAT		2160	
142									
143	CAAGGAAAGA	ATGAGTCTAA	TATCAAGCCT	GTACAGACAG	TTAATATCAC	TGCAGGCTTT		2220	
144									
145	CCTGTGGTTG	GTCAGAAAGA	TAAGCCAGTT	GATAATGCCA	AATGTAGTAT	CAAAGGAGGC		2280	
146									
147	TCTAGGTTTT	GTCTATCATC	TCAGTTCAGA	GGCAACGAAA	CTGGACTCAT	TACTCCAAAT		2340	
148									
149	AAACATGGAC	TTTTTACAAA	CCCATATCGT	ATACCACCAC	TTTTTCCCAT	CAAGTCATTT		2400	
150									
151	GTAAAACTA	AATGTAAGAA	AAATCTGCTA	GAGGAAAAC	TTGAGGAACA	TTCAATGTCA		2460	
152									

RAW SEQUENCE LISTING PATENT APPLICATION US/08/289,221

DATE: 08/19/94
TIME: 18:06:11

INPUT SET: S8412.raw

153	CCTGAAAGAG	AAATGGGAAA	TGAGAACATT	CCAAGTACAG	TGAGCACAAT	TAGCCGTAAT	2520
154							
155	AACATTAGAG	AAAATGTTTT	TAAAGAAGCC	AGCTCAAGCA	ATATTAATGA	AGTAGGTTCC	2580
156							
157	AGTACTAATG	AAGTGGGCTC	CAGTATTAAT	GAAATAGGTT	CCAGTGATGA	AAACATTCAA	2640
158							
159	GCAGAACTAG	GTAAGAACAG	AGGGCCAAAA	TTGAATGCTA	TGCTTAGATT	AGGGGTTTTG	2700
160							
161	CAACCTGAGG	TCTATAAACA	AAGTCTTCCT	GGAAGTAATT	GTAAGCATCC	TGAAATAAAA	2760
162							
163	AAGCAAGAAT	ATGAAGAAGT	AGTTCAGACT	GTTAATACAG	ATTTCTCTCC	ATCTCTGATT	2820
164							
165	TCAGATAACT	TAGAACAGCC	TATGGGAAGT	AGTCATGCAT	CTCAGGTTTG	TTCTGAGACA	2880
166							
167	CCTGATGACC	TGTTAGATGA	TGGTGAAATA	AAGGAAGATA	CTAGTTTTGC	TGAAATGACA	2940
168							
169	TTAAGGAAAAG	TTCTGCTGTT	TTTAGCAAAA	GCGTCCAGAG	GAGAGCTTAG	CAGGGTCCTA	3000
170							
171	GCCCTTTTAC	CCATACACAT	TTGGCTCAGG	GTTACCGAAG	AGGGGCCAAG	AAATTAGAGT	3060
172							
173	CCTCAGAAGA	GAACCTATCT	AGTGAGGATG	AAGAGCTTCC	CTGCTTCCAA	CACTTGTTAT	3120
174							
175	TTGGTAAAGT	AAACAATATA	CCTTCTCAGT	CTACTAGGCA	TAGCACCGTT	GCTACCGAGT	3180
176							
177	GTCTGTCTAA	GAACACAGAG	GAGAATTTAT	TATCATTGAA	GAATAGCTTA	AATGACTGCA	3240
178							
179	GTAACCAGGT	AATATTGGCA	AAGGCATCTC	AGGAACATCA	CCTTAGTGAG	GAAACAAAAT	3300
180							
181	GTTCTGCTAG	CTTGTTTTCT	TCACAGTGCA	GTGAATTGGA	AGACTTGACT	GCAAATACAA	3360
182							
183	ACACCCAGGC	TCCTTTCTTG	ATTGGTTCTT	CCAAACAAAT	GAGGCATCAG	TCTGAAAGCC	3420
184							
185	AGGGAGTTGG	TCTGAGTGAC	AAGGAATTGG	TTTCAGATGA	TGAAGAAAGA	GGAACGGGCT	3480
186							
187	TGGAAGAAAA	TAATCAAGAA	GAGCAAAGCA	TGGATTCAAA	CTTAGGTGAA	GCAGCATCTG	3540
188							
189	GGTGTGAGAG	TGAAACAAGC	GTCTCTGAAG	ACTGCTCAGG	GCTATCCTCT	CAGAGTGACA	3600
190							
191	TTTAAACCAC	TCAGCAGAGG	GATACCATGC	AACATAACCT	GATAAAGCTC	CAGCAGGAAA	3660
192							
193	TGGCTGAACT	AGAAGCTGTG	TTAGAACAGC	ATGGGAGCCA	GCCTTCTAAC	AGCTACCCTT	3720
194							
195	CCATCATAAG	TGACTCTTCT	GCCCTTGAGG	ACCTGCCGAA	TCCAGAACAA	AGCACATCAG	3780
196							
197	AAAAAGTATT	AACTTCACAG	AAAAGTAGTG	AATACCCTAT	AAGCCAGAAT	CCAGAAGGCC	3840
198							
199	TTCTGCTGAC	AAGTTTGAGG	TGTCTGCAGA	TAGTTCTACC	AGTAAAAATA	AAGAACCAGG	3900
200							
201	AGTGGAAGAG	TCATCCCCCT	CTAAATGCCC	ATCATTAGAT	GATAGGTGGT	ACATGCACAG	3960
202							
203	TTGCTCTGGG	AGTCTTCAGA	ATAGAAACTA	CCCACCTCAA	GAGGAGCTCA	TTAAGGTTGT	4020
204							
205	TGATGTGGAG	GAGCAACAGC	TGGAAGAGTC	TGGGCCACAC	GATTTGACGG	AAACATCTTA	4080

206	2001 BETA-SOFT-100							
207	CTTGCCAAGG	CAAGATCTAG	AGGGAACCCC	TTACCTGGAA	TCTGGAATCA	GCCTCTTCTC		4140
208								
209	TGATGACCCT	GAATCTGATC	CTTCTGAAGA	CAGAGCCCCA	GAGTCAGCTC	GTGTTGGCAA		4200
210								
211	CATACCATCT	TCAACCTCTG	CATTGAAAGT	TCCCCAATTG	AAAGTTGCAG	AATCTGCCCCA		4260
212								
213	GAGTCCAGCT	GCTGCTCATA	CTACTGATAC	TGCTGGGTAT	AATGCAATGG	AAGAAAGTGT		4320
214								
215	GAGCAGGGAG	AAGCCAGAAT	TGACAGCTTC	AACAGAAAGG	GTCAACAAAA	GAATGTCCAT		4380
216								
217	GGTGGTGTCT	GGCCTGACCC	CAGAAGAATT	TATGCTCGTG	TACAAGTTTG	CCAGAAAAACA		4440
218								
219	CCACATCACT	TTAACTAATC	TAATTACTGA	AGAGACTACT	CATGTTGTTA	TGAAAACAGA		4500
220								
221	TGCTGAGTTT	GTGTGTGAAC	GGACACTGAA	ATATTTTCTA	GGAATTGCGG	GAGGAAAATG		4560
222								
223	GGTAGTTAGC	TATTTCTGGG	TGACCCAGTC	TATTAAAGAA	AGAAAAATGC	TGAATGAGCA		4620
224								
225	TGATTTTGAA	GTCAGAGGAG	ATGTGGTCAA	TGGAAGAAAC	CACCAAGGTC	CAAAGCGAGC		4680
226								
227	AAGAGAATCC	CAGGACAGAA	AGATCTTCAG	GGGGCTAGAA	ATCTGTTGCT	ATGGGCCCTT		4740
228								
229	CACCAACATG	CCCACAGATC	AACTGGAATG	GATGGTACAG	CTGTGTGGTG	CTTCTGTGGT		4800
230								
231	GAAGGAGCTT	TCATCATTCA	CCCTTGGCAC	AGGTGTCCAC	CCAATTGTGG	TTGTGCAGCC		4860
232								
233	AGATGCCTGG	ACAGAGGACA	ATGGCTTCCA	TGCAATTGGG	CAGATGTGTG	AGGCGCCTGT		4920
234								
235	GGTGACCCGA	GAGTGGGTGT	TGGACAGTGT	AGCACTCTAC	CAGTGCCAGG	AGCTGGACAC		4980
236								
237	CTACCTGATA	CCCCAGATCC	CCCACAGCCA	CTACTGACTG	CAGCCAGCCA	CAGGTACAGA		5040
238								
239	GCCACAGGAC	CCCAAGAATG	AGCTTACAAA	GTGGCCTTTC	CAGGCCCTGG	GAGCTCCTCT		5100
240								
241	CACTCTTCAG	TCCTTCTACT	GTCCTGGCTA	CTAAATATTT	TATGTACATC	AGCCTGAAAA		5160
242								
243	GGACTTCTGG	CTATGCAAGG	GTCCCTTAAA	GATTTTCTGC	TTGAAGTCTC	CCTTGGAAAT		5220

GeneDX 1022, pg. 132

PAGE: 1

SEQUENCE VERIFICATION REPORT
PATENT APPLICATION *US/08/289,221*

DATE: 08/19/94
TIME: 18:06:21

INPUT SET: S8412.raw

Line	Error	Original Text
33	Wrong application Serial Number	(A) APPLICATION NUMBER: US



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
--------------------	-------------	-----------------------	------------------------

08/289,221

08/12/94

SKOLNICK

M

248841093471

08A1/0826

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 NEW YORK AVENUE, NW
STE. 1000
WASHINGTON, DC 20005

0000

DATE MAILED:

08/26/94

NOTICE TO FILE MISSING PARTS OF APPLICATION
FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 130.00 for large entities or \$ 65.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 3146.00

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

1. ☒ The statutory basic filing fee is: ☒ missing ☐ insufficient. Applicant as a ☒ large entity ☐ small entity, must submit \$ 710.00 to complete the basic filing fee.

2. ☒ Additional claim fees of \$ 2306.00 as a ☒ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.

3. ☒ The oath or declaration:
☒ is missing.
☐ does not cover items omitted at time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.

4. ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.

5. ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.

6. ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:

_____ An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.

7. ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.

8. ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).

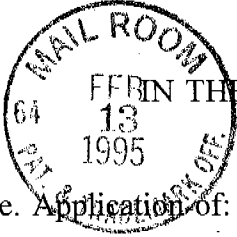
9. ☐ Your filing receipt was mailed in error because your check was returned without payment.

10. ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with Sequence Rules 37 CFR 1.821-1.825.

11. ☐ Other

Direct the response and any questions about this notice to, Attention: Application Processing Division, Special Processing and Correspondence Branch (703) 308-1202.

A copy of this notice MUST be returned with the response.



In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

TRANSMITTAL LETTER

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

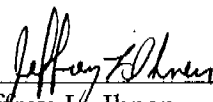
Transmitted herewith in response to the Notice To File Missing Parts mailed 26 August 1994 for the above-identified application are the following papers:

1. A copy of the signed Declarations and Powers of Attorney, the originals of which are attached to Applicants' Petition Under 37 CFR §1.48 to Add Joint Inventors;
2. Petition Under 37 CFR §1.48 to Add Joint Inventors, including attached Verified Statements of Facts, original Declarations and Powers of Attorney and check for \$130.00 for the petition fee, with authorization to charge any discrepancies to our deposit account;
3. Two chsecks totaling \$4,308.00, covering the filing fee (\$3,068.00), surcharge (\$130.00), and a portion of the extension fee (\$1,110.00);
4. A Petition for Extension of Four (4) Months, in triplicate, with authorization to charge Deposit Account 22-0261 for the remainder of the extension fee (\$250.00); and
5. Copy of Form PTO-1533.

-2-

If any discrepancies in fees are noted, the same are to be charged or credited to Deposit Account No. 22-0261. This transmittal letter is submitted in triplicate.

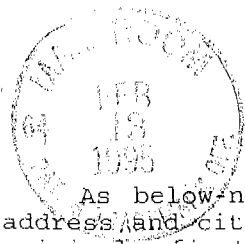
Respectfully submitted,



Jeffrey L. Ihnen
Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., Suite 1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347-1
Dated: 13 February 1995



DECLARATION AND POWER OF ATTORNEY

As below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Priority Claimed</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or
First Inventor

Mark H. SKOLNICK

Inventor's Signature

Mark Skolnick

Date of Signature

Feb. 9, 1995

Residence Address

460 Northmount Way
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Second
Inventor

David E. GOLDGAR

Inventor's Signature

Date of Signature

Residence Address

2707 East Kelly Lane
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Third
Inventor

Yoshio MIKI

Inventor's Signature

Yoshio Miki

Date of Signature

Feb. 9, 1995

Residence Address

40 South 900 East
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

JAPAN

vided information from its research records describing the identification of fragments of coding sequences by Wiseman and Futreal from genetic materials obtained from Myriad. After a thorough analysis was made of all of the pertinent research records of the University and Myriad and of the information provided by NIEHS, it was determined that Wiseman and Futreal had been involved in the identification of two DNA fragments which are part of the breast cancer gene. After the facts were completely analyzed, it was recognized that Wiseman and Futreal are joint inventors as to a fragment claim and had been inadvertently omitted, without any deceptive intent, from the application as filed under 37 CFR §1.53(d) and should be properly joined as coinventors of the claimed subject matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Feb 9, 1995
Date

Mark H. Skolnick
Mark H. Skolnick

Date

David E. Goldgar

2/9/95
Date

Yoshio Miki
Yoshio Miki

2/9/95
Date

Jeff Swenson
Jeff Swenson

2/9/95
Date

Alexander Kamb
Alexander Kamb

2.9.95
Date

Keith D. Harshman
Keith D. Harshman

-4-

Date

Donna M. Shattuck-Eidens

Date

Sean V. Tavtigian

g:\pat\myriad\pto\papers\brca1\221ver.stm



DECLARATION AND POWER OF ATTORNEY

As below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>	<u>Priority Claimed</u>
<u>Appln. No.</u> <u>Country</u> <u>Date Filed</u>	<u>Yes</u> <u>No</u>
<u>Appln. No.</u> <u>Country</u> <u>Date Filed</u>	<u>Yes</u> <u>No</u>
<u>Appln. No.</u> <u>Country</u> <u>Date Filed</u>	<u>Yes</u> <u>No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or, PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

4 I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or First Inventor

1-00 Mark H. SKOLNICK

Inventor's Signature

Mark Skolnick

Date of Signature

Feb. 9, 1995

Residence Address

460 Northmount Way
Salt Lake City, Utah 84103
UT

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Second Inventor

David E. GOLDFAR

Inventor's Signature

David E. Goldfar

Date of Signature

Residence Address

2707 East Kelly Lane
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Third Inventor

3-0 Yoshio MIKI

Inventor's Signature

Yoshio Miki

Date of Signature

Feb. 9, 1995

Residence Address

40 South 900 East
Salt Lake City, Utah 84102
UT

Post Office Address

SAME

Country of Citizenship

JAPAN

Full Name of Fourth
Inventor

Jeff SWENSON

Inventor's Signature

Jeff Swenson

Date of Signature

2/9/95

Residence Address

~~745 West Creek Bend, #2807~~

~~Salt Lake City, Utah 84117~~

1210 E Lorraine Dr

SLC, UT 84106

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Fifth
Inventor

Alexander KAMB

Inventor's Signature

Alexander Kamb

Date of Signature

2/9/95

Residence Address

1103 East 600 South

Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Sixth
Inventor

Keith D. HARSHMAN

Inventor's Signature

Date of Signature

Residence Address

159 North F Street

Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Seventh
Inventor

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Donna M. Shattuck-Eidens

Date of Signature

2/10/95

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

Sean V. TAVTIGIAN

Inventor's Signature

SVI

Date of Signature

2/9/85

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Tenth
Inventor

P. Andrew FUTREAL

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA



DECLARATION AND POWER OF ATTORNEY

As [redacted] inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original first and sole inventor (if only one name is listed below) or an original first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Priority Claimed</u>	
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or First Inventor	<u>Mark H. SKOLNICK</u>
Inventor's Signature	_____
Date of Signature	_____
Residence Address	<u>460 Northmount Way</u> <u>Salt Lake City, Utah 84103</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>USA</u>
200 Full Name of Second Inventor	<u>David E. GOLDGAR</u>
Inventor's Signature	<u>David E. Goldgar by Jeffrey L. Ihnen</u>
Date of Signature	<u>13 February 1995</u>
Residence Address	<u>2707 East Kelly Lane</u> <u>Salt Lake City, Utah 84117</u> <u>U7</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>USA</u>
Full Name of Third Inventor	<u>Yoshio MIKI</u>
Inventor's Signature	_____
Date of Signature	_____
Residence Address	<u>40 South 900 East</u> <u>Salt Lake City, Utah 84102</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>JAPAN</u>

Full Name of Fourth
Inventor

Jeff SWENSON

Inventor's Signature

Date of Signature

Residence Address

745 West Creek Bend, #2807
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Fifth
Inventor

Alexander KAMB

Inventor's Signature

Date of Signature

Residence Address

1103 East 600 South
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Sixth
Inventor

Keith D. HARSHMAN

Inventor's Signature

Date of Signature

Residence Address

159 North F Street
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Seventh
Inventor

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Date of Signature

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

Sean V. TAVTIGIAN

Inventor's Signature

Date of Signature

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Tenth
Inventor

P. Andrew FUTREAL

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA



DECLARATION AND POWER OF ATTORNEY

As below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Prior Claimed</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

-2-

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or
First Inventor

Mark H. SKOLNICK

Inventor's Signature

Date of Signature

Residence Address

460 Northmount Way
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Second
Inventor

David E. GOLDGAR

Inventor's Signature

Date of Signature

Residence Address

2707 East Kelly Lane
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Third
Inventor

Yoshio MIKI

Inventor's Signature

Date of Signature

Residence Address

40 South 900 East
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

JAPAN

FEB 09 '95 16:15 FR UENABLE BAETJER

202 962 830 TO 999#19195413720# P.04/17

-3-

Full Name of Fourth
Inventor

Jeff SWENSON

Inventor's Signature

Date of Signature

Residence Address

745 West Creek Bend, #2807
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Fifth
Inventor

Alexander KAMB

Inventor's Signature

Date of Signature

Residence Address

1103 East 600 South
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Sixth
Inventor

Keith D. HARSHMAN

Inventor's Signature

Date of Signature

Residence Address

159 North F Street
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

FEB 09 '95 16:15 FR VENABLE BAETJER

202 962 8300 TO 999#19195413720# P.05/17

-4-

Full Name of Seventh
Inventor

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Date of Signature

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

Sean V. TAVTIGIAN

Inventor's Signature

Date of Signature

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

515 Woodwinds Drive
Durham, NC 27713

Post Office Address

SAME

Country of Citizenship

USA

FEB 09 '95 16:16 FR VENABLE BAETJER

202 862 8300

999#19195413720# P.07/17

-5-

Full Name of Tenth
Inventor

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

Country of Citizenship

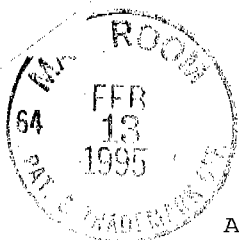
10-00
P. Andrew FUTREAL

[Signature]
2/9/95

220 East Markham Ave
Durham NC 27701

SAME

USA



DECLARATION AND POWER OF ATTORNEY

As below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Priority Claimed</u>	
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes</u>	<u>No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or
First Inventor

Mark H. SKOLNICK

Inventor's Signature

Date of Signature

Residence Address

460 Northmount Way
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Second
Inventor

David E. GOLDGAR

Inventor's Signature

David E. Goldgar by Jeffrey L. Ihnen

Date of Signature

13 February 1995

Residence Address

2707 East Kelly Lane
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Third
Inventor

Yoshio MIKI

Inventor's Signature

Date of Signature

Residence Address

40 South 900 East
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

JAPAN

Full Name of Fourth
Inventor

4-00
Jeff SWENSON

Inventor's Signature

Jeff Swenson

Date of Signature

2/9/95

Residence Address

~~745 West Creek Bend, #2807~~ 1210 E Lorraine Dr
~~Salt Lake City, Utah 84117~~ SLC, UT 84106

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Fifth
Inventor

5-00
Alexander KAMB

Inventor's Signature

Alexander Kamb

Date of Signature

2/9/95

Residence Address

1103 East 600 South
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Sixth
Inventor

Keith D. HARSHMAN

Inventor's Signature

Keith D. Harshman

Date of Signature

Residence Address

159 North F Street
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

mlr.

Full Name of Seventh
Inventor

7-W

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Donna M. Shattuck Eiden

Date of Signature

2/10/95

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108
UT

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

8-W

Sean V. TAVTIGIAN

Inventor's Signature

SVI

Date of Signature

2/9/85

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103
UT

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

Country of Citizenship

SAME

USA

mtt.

-5-

Full Name of Tenth
Inventor

P. Andrew FUTREAL

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA



DECLARATION AND POWER OF ATTORNEY

As below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Priority Claimed</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

-2-

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or
First Inventor

Mark H. SKOLNICK

Inventor's Signature

Date of Signature

Residence Address

460 Northmount Way
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Second
Inventor

David E. GOLDBAR

Inventor's Signature

Date of Signature

Residence Address

2707 East Kelly Lane
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Third
Inventor

Yoshio MIKI

Inventor's Signature

Date of Signature

Residence Address

40 South 900 East
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

JAPAN

Full Name of Fourth
Inventor

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

Country of Citizenship

Full Name of Fifth
Inventor

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

Country of Citizenship

Full Name of Sixth
Inventor

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

Country of Citizenship

JEFF SWENSON

Jeff Swenson
2/9/95

~~245 West Creek Bend, #2807~~ 1210 E Lorraine Dr
~~Salt Lake City, Utah 84117~~ SL, UT 84106

SAME

USA

Alexander KAMB

Alexander Kamb
2/9/95

1103 East 600 South
Salt Lake City, Utah 84102

SAME

USA

6-00 Keith D. HARSHMAN

Keith D. Harshman
2.9.95

159 North F Street
Salt Lake City, Utah 84103

SAME

USA

-4-

Full Name of Seventh
Inventor

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Date of Signature

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

Sean V. TAVTIGIAN

Inventor's Signature

Date of Signature

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

Roger Wiseman
2/9/95
515 Woodwinds Drive
Durham, NC 27713

Post Office Address

SAME

Country of Citizenship

USA

B 09'95 16:16 FR VENABLE BREYER

202 562 8300 TO 5534751334751204 1.01.11

-5-

Full Name of Tenth
Inventor

P. Andrew FUTREAL

Inventor's Signature

P. Andrew Futreal

Date of Signature

2/9/95

Residence Address

220 East Markham Ave
Durham NC 27701

Post Office Address

SAME

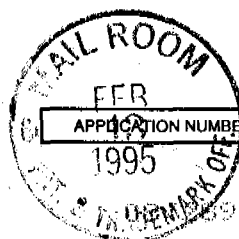
Country of Citizenship

USA



30-101
130-105
338-103
A/n

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231



APPLICATION NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
--------------------	-------------	-----------------------	------------------------

09,221	08/12/94	SKOLNICK	M 248841093471
--------	----------	----------	----------------

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 NEW YORK AVENUE, NW
STE. 1000
WASHINGTON, DC 20005

08A1/0826

#4

DATE MAILED: 08/26/94

NOTICE TO FILE MISSING PARTS OF APPLICATION FILING DATE GRANTED

An Application Number and Filing Date have been assigned to this application. However, the items indicated below are missing. The required items and fees identified below must be timely submitted **ALONG WITH THE PAYMENT OF A SURCHARGE** for items 1 and 3-6 only of \$ 120.00 for large entities or \$ 60.00 for small entities who have filed a verified statement claiming such status. The surcharge is set forth in 37 CFR 1.16(e).

If all required items on this form are filed within the period set below, the total amount owed by applicant as a ☒ large entity, ☐ small entity (verified statement filed), is \$ 3146.00

Applicant is given **ONE MONTH FROM THE DATE OF THIS LETTER, OR TWO MONTHS FROM THE FILING DATE** of this application, **WHICHEVER IS LATER**, within which to file all required items and pay any fees required above to avoid abandonment. Extensions of time may be obtained by filing a petition accompanied by the extension fee under the provisions of 37 CFR 1.136(a).

- ☒ The statutory basic filing fee is: ☒ missing ☐ insufficient. Applicant as a ☒ large entity ☐ small entity, must submit \$ 710.00 to complete the basic filing fee.
- ☒ Additional claim fees of \$ 2306.00 as a ☒ large entity, ☐ small entity, including any required multiple dependent claim fee, are required. Applicant must submit the additional claim fees or cancel the additional claims for which fees are due.
- ☒ The oath or declaration:
☒ is missing.
☐ does not cover items omitted at time of execution.

An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date is required.

- ☐ The oath or declaration does not identify the application to which it applies. An oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature(s) to the oath or declaration is/are: ☐ missing; ☐ by a person other than the inventor or a person qualified under 37 CFR 1.42, 1.43, or 1.47. A properly signed oath or declaration in compliance with 37 CFR 1.63, identifying the application by the above Application Number and Filing Date, is required.
- ☐ The signature of the following joint inventor(s) is missing from the oath or declaration:

An oath or declaration listing the names of all inventors and signed by the omitted inventor(s), identifying this application by the above Application Number and Filing Date, is required.
- ☐ The application was filed in a language other than English. Applicant must file a verified English translation of the application and a fee of \$ _____ under 37 CFR 1.17(k), unless this fee has already been paid.
- ☐ A \$ _____ processing fee is required since your check was returned without payment. (37 CFR 1.21(m)).
- ☐ Your filing receipt was mailed in error because your check was returned without payment.
- ☐ The application does not comply with the Sequence Rules. See attached Notice to Comply with

02 Sequence Rules 37 CFR 1.821-1.825. 1 101 609.00 CK

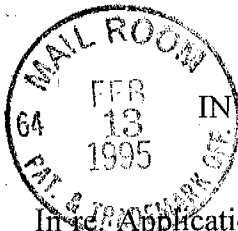
020 AA 02/24/95 00289221 1 101 121.00 CK
020 AA 02/24/95 00289221 1 105 130.00 CK
020 AA 02/24/95 00289221 1 103 2,330.00 CK

Direct the response and any questions about this notice to, Attention: Application Processing Division, Special Processing and Correspondence Branch (703) 308-1202.

A copy of this notice MUST be returned with the response.

130-122

A/n
#4



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

PETITION UNDER 37 CFR §1.48 TO ADD JOINT INVENTORS

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

It is respectively petitioned that the above-identified application, filed under 37 CFR §1.53(d), be amended to add as joint inventors the following individuals:

Roger W. WISEMAN
P. Andrew FUTREAL

Through error and without any deceptive intention, the application was made in the names of Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens and Sean V. Tavtigian as coinventors, which is apparent from the Verified Statement of Facts submitted concurrently herewith.

In support of this Petition, Applicants submit the following documents:

- (1) A Verified Statement of Facts by the inventors named in the application: Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens and Sean V. Tavtigian;
- (2) A Declaration and Power of Attorney under 37 CFR §1.63 by all of the *actual* joint inventors;
- (4) A check in the amount of \$130.00 for the fee required under 37 CFR §1.17(h). If any discrepancies in fee are noted, the same

020 AA 02/24/95 08289221

1 122

130.00 CK

should be charged or credited to Deposit Account No. 22-0261.
Three copies of this Petition are enclosed.

As is evident from the Verified Statement of Facts, the present invention is related to three applications, in which Mark Skolnick, David Goldgar, Yoshio Miki and Jeff Swenson, all of the University of Utah are co-inventors and a fourth application, in which Mark Skolnick, David Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith Harshman and Donna Shattuck-Eidens (the latter three of Myriad Genetics) are co-inventors. Roger Wiseman and Andrew Futreal were *not* coinventors on any of these applications.

It is clear from the Verified Statement that the decision to prepare and file the present application was made on 10 August 1994 with the filing to be made on 12 August 1994. Inventorship of the breast cancer gene was briefly discussed during the 10 August 1994 meeting. Based on the available information, it was determined that Roger Wiseman and Andrew Futreal were not joint inventors of the breast cancer gene, since they did not develop the entire gene sequence and had not discovered any mutations in the gene.


On 5 October 1994, following the filing of the present application, NIEHS claimed that Wiseman and Futreal were joint inventors of the application, since claim 3 in the application was directed to fragments of the breast cancer gene. On 28 November 1994 NIEHS provided information from its research records describing the identification of fragments of coding sequences by Wiseman and Futreal from materials obtained from Myriad. After a thorough analysis was made of all of the pertinent research records of the University of Utah and Myriad Genetics and of the information provided by NIEHS, it was determined that Wiseman and Futreal had been involved in the identification of two DNA fragments which are part of the breast cancer gene. After the facts were completely analyzed, it was recognized that Wiseman and Futreal are joint inventors as to a fragment claim and had been inadvertently omitted, without any deceptive intent, from the application as filed. As soon as it became apparent that Wiseman and Futreal were properly joint inventors, and in preparation for filing missing parts to the above application, this Petition was diligently made. See *Koret v. Hoskins*, 84 USPQ 461 (CCPA 1950). Accordingly, this Petition is timely.

Further, the statutes and rules governing inventorship conversion are remedial in nature and should be liberally construed in order that the error noted herein may be rectified. *Patterson & Lissner v. Hauck*, 144 USPQ 481 (CCPA 1965); *In re Schmidt*, 130 USPQ 404 (CCPA 1961); *Fritsch v. Lin*, 21 USPQ2d 1731 (BPAI 1991).

-3-

Thus, it is submitted that Wiseman and Futreal are properly joint inventors of the presently claimed subject matter, and that their omission from the application as filed was without deceptive intent. It is requested that the inventorship of this application be amended to add Roger W. Wiseman and P. Andre Futreal as coinventors.

Respectfully submitted,



Jeffrey L. Ilmen
Registration No. 28,957

JLI:ch

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347-1
Dated: 13 February 1995

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

VERIFIED STATEMENT OF FACTS

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens and Sean V. Tavtigian declare that:

(1) The invention described and claimed in the above-identified application was developed in part by us and in part by Roger W. Wiseman and P. Andrew Futreal.

(2) Mark Skolnick, David Goldgar, Yoshio Miki and Jeff Swenson are employees of the University of Utah (University) and have been conducting research to identify and characterize a breast cancer gene on human chromosome 17 since at least January 1991. This research has led to three earlier-filed related applications of which Skolnick, Goldgar, Miki and Swenson are coinventors.

(3) Mark Skolnick and his fellow University researchers began collaborating with Myriad Genetics, Inc. (Myriad) and its researchers on May 1, 1992 to intensify the search for the breast cancer gene. As a result of this collaborative effort, Mark Skolnick, David Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith Harshman and Donna Shattuck-Eidens identified a gene which was disclosed and claimed in a fourth, earlier-filed related ap-

plication as a breast cancer gene. This fourth related application also disclosed other candidate DNA sequences.

(4) Mark Skolnick and his fellow University researchers began collaborating with Roger Wiseman and his fellow researchers at the National Institute of Environmental Health Sciences (NIEHS) in April 1992 to intensify the search for the breast cancer gene. The researchers at Myriad were included in this collaboration, as well as researchers at other universities and companies.

(5) Mark Skolnick, Alexander Kamb or Donna Shattuck-Eidens periodically communicated research results with Jeffrey Ihnen, patent counsel to the University and Myriad, to update him on the results of their research. In early August 1994, Jeffrey Ihnen was advised that the gene identified in the fourth related application as being the breast cancer gene was not thought to be the correct gene and that about 70% of the DNA coding sequence for the correct gene had been determined. During a meeting on 10 August 1994 with Jeffrey Ihnen, it was decided to proceed with the filing of an application to claim the gene sequence which was then known as well as the predisposing mutations which had been discovered. It was also decided that the application would be filed on 12 August 1994.

(6) Inventorship of the breast cancer gene was briefly discussed during the 10 August 1994 meeting. Based on the available information, it was determined that the inventorship for the breast cancer gene was as set forth in the transmittal letter for this application. That is, it was determined that Roger Wiseman and Andrew Futreal were not joint inventors of the breast cancer gene, since they did not develop the entire gene sequence (then known) and had not discovered any mutations in the gene.

(7) The application was filed on 12 August 1994 without a Declaration, pursuant to the provisions of 37 CFR §1.53(d), naming us as joint inventors.

(8) On 5 October 1994, following the filing of the present application, NIEHS claimed that Wiseman and Futreal were joint inventors of the application, since claim 3 in the application was directed to fragments of the breast cancer gene. On 28 November 1994 NIEHS pro-

vided information from its research records describing the identification of fragments of coding sequences by Wiseman and Futreal from genetic materials obtained from Myriad. After a thorough analysis was made of all of the pertinent research records of the University and Myriad and of the information provided by NIEHS, it was determined that Wiseman and Futreal had been involved in the identification of two DNA fragments which are part of the breast cancer gene. After the facts were completely analyzed, it was recognized that Wiseman and Futreal are joint inventors as to a fragment claim and had been inadvertently omitted, without any deceptive intent, from the application as filed under 37 CFR §1.53(d) and should be properly joined as coinventors of the claimed subject matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

13 February 1995
Date

Mark H. Skolnick

David E. Goldgar by Jeffrey Allen
David E. Goldgar

Date

Yoshio Miki

Date

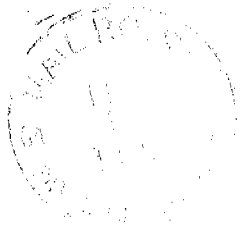
Jeff Swenson

Date

Alexander Kamb

Date

Keith D. Harshman



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

VERIFIED STATEMENT OF FACTS

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens and Sean V. Tavtigian declare that:

(1) The invention described and claimed in the above-identified application was developed in part by us and in part by Roger W. Wiseman and P. Andrew Futreal.

(2) Mark Skolnick, David Goldgar, Yoshio Miki and Jeff Swenson are employees of the University of Utah (University) and have been conducting research to identify and characterize a breast cancer gene on human chromosome 17 since at least January 1991. This research has led to three earlier-filed related applications of which Skolnick, Goldgar, Miki and Swenson are coinventors.

(3) Mark Skolnick and his fellow University researchers began collaborating with Myriad Genetics, Inc. (Myriad) and its researchers on May 1, 1992 to intensify the search for the breast cancer gene. As a result of this collaborative effort, Mark Skolnick, David Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith Harshman and Donna Shattuck-Eidens identified a gene which was disclosed and claimed in a fourth, earlier-filed related ap-

plication as a breast cancer gene. This fourth related application also disclosed other candidate DNA sequences.

(4) Mark Skolnick and his fellow University researchers began collaborating with Roger Wiseman and his fellow researchers at the National Institute of Environmental Health Sciences (NIEHS) in April 1992 to intensify the search for the breast cancer gene. The researchers at Myriad were included in this collaboration, as well as researchers at other universities and companies.

(5) Mark Skolnick, Alexander Kamb or Donna Shattuck-Eidens periodically communicated research results with Jeffrey Ihnen, patent counsel to the University and Myriad, to update him on the results of their research. In early August 1994, Jeffrey Ihnen was advised that the gene identified in the fourth related application as being the breast cancer gene was not thought to be the correct gene and that about 70% of the DNA coding sequence for the correct gene had been determined. During a meeting on 10 August 1994 with Jeffrey Ihnen, it was decided to proceed with the filing of an application to claim the gene sequence which was then known as well as the predisposing mutations which had been discovered. It was also decided that the application would be filed on 12 August 1994.

(6) Inventorship of the breast cancer gene was briefly discussed during the 10 August 1994 meeting. Based on the available information, it was determined that the inventorship for the breast cancer gene was as set forth in the transmittal letter for this application. That is, it was determined that Roger Wiseman and Andrew Futreal were not joint inventors of the breast cancer gene, since they did not develop the entire gene sequence (then known) and had not discovered any mutations in the gene.

(7) The application was filed on 12 August 1994 without a Declaration, pursuant to the provisions of 37 CFR §1.53(d), naming us as joint inventors.

(8) On 5 October 1994, following the filing of the present application, NIEHS claimed that Wiseman and Futreal were joint inventors of the application, since claim 3 in the application was directed to fragments of the breast cancer gene. On 28 November 1994 NIEHS pro-

vided information from its research records describing the identification of fragments of coding sequences by Wiseman and Futreal from genetic materials obtained from Myriad. After a thorough analysis was made of all of the pertinent research records of the University and Myriad and of the information provided by NIEHS, it was determined that Wiseman and Futreal had been involved in the identification of two DNA fragments which are part of the breast cancer gene. After the facts were completely analyzed, it was recognized that Wiseman and Futreal are joint inventors as to a fragment claim and had been inadvertently omitted, without any deceptive intent, from the application as filed under 37 CFR §1.53(d) and should be properly joined as coinventors of the claimed subject matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Feb 9, 1995
Date

Mark H. Skolnick
Mark H. Skolnick

Date

David E. Goldgar

2/9/95
Date

Yoshio Miki
Yoshio Miki

2/9/95
Date

Jeff Swenson
Jeff Swenson

2/9/95
Date

Alexander Kamb
Alexander Kamb

Date

Keith D. Harshman

2/10/95
Date

Donna M. Shattuck-Eidens
Donna M. Shattuck-Eidens

2/9/95
Date

SV
Sean V. Tavtigian

g:\pat\myriad\pto\papers\brca1\221ver.stm



1,110-1'8

A/n

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

PETITION FOR EXTENSION OF TIME

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

In the matter of the above-identified application, petition is hereby made for an extension of time of four (4) months in which to respond.

A check is attached to the transmittal letter which includes \$1,110.00 for a portion of the four month extension fee. Please charge the remainder of the petition fee of \$250.00, and any noted discrepancies in fee, to Deposit Account No. 22-0261. This Petition is submitted in triplicate.

Respectfully submitted,

Jeffrey L. Ihnen

Registration No. 28,957

JLI:ch

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005

(202) 962-4810

CS14124 03/01/95 08289221

22-0261 140 118

250.00CH

Atty. Docket No. 24884-109347-1

Dated: 13 February 1995

020 AA 02/24/95 08289221

1 118 1,110.00 CK



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 5

COPY MAILED

APR 18 1995

**SPECIAL PROGRAMS OFFICE
DAC FOR PATENTS**

Venable, Baetjer, Howard & Civiletti
1201 New York Avenue, N.W.
Suite 1000
Washington, D.C. 20005

In re Application of	:	
Mark H. Skolnick, et al	:	REQUIREMENT FOR INFORMATION
Application No. 08/289,221	:	RE PETITION UNDER 37 CFR
Filed: August 12, 1994	:	1.48(a)
For: 17q-LINKED BREAST AND	:	
OVARIAN CANCER	:	
SUSCEPTABILITY GENE	:	

This is in response to a petition under 37 CFR 1.48(a) filed February 13, 1995, requesting that the name of the inventors in the application be corrected. The petition is being decided by the undersigned rather than in the examining group so that application processing, including the issuance of a filing receipt, may proceed with the actual inventors being named in the application.

The instant application was filed on August 12, 1994, as a CIP of prior Application No. 08/246,602 filed May 20, 1994 (pending), pursuant to 37 CFR 1.53(b) without the filing fees, without an executed oath or declaration under 37 CFR 1.63. The prior named as the inventors: Skolnick, Golfgar, Miki and Swenson whereas instant application named as the inventors: Skolnick, Goldgar, Miki, Swenson, Kamb, Harshman, Shattuck-Eidens and Tavgitian.

Application Processing Division mailed a Notice to File Missing Parts of Application under 37 CFR 1.53(b) on August 26, 1994, requiring an oath or declaration in compliance with 37 CFR 1.63, the filing fees and a surcharge for their late submission.

In response to the Notice to File Missing Parts of Application, applicants timely filed on February 13, 1995 (with a petition and fee for a four month extension of time under 37 CFR 1.136(a)), inter alia, the instant \$ 1.48(a) petition and fee, the filing fees, the surcharge, three combined Declarations under 37 CFR 1.63 and Powers of Attorney and two verified statements of facts by the original named inventors.

The petition and verified statements of facts state that an error occurred in failing to name Wiseman and Futreal as co-inventors, that the instant application is directed to the breast cancer gene and the inventorship was set forth based thereon and that after the application was filed it was pointed out that claim 3 is directed to fragments of the breast cancer gene for which Wiseman and Futreal are joint inventors.

37 CFR 1.48(a) requires the written consent of any existing assignee. In view of the references to the University of Utah, Myriad Genetics, Inc. and the National Institute of Environmental Health Sciences, clarification is requested as to the existence of any assignee requiring its consent.

Accordingly, the petition under § 1.48(a) to correct the inventorship cannot be granted at this time.

Applicants are given TWO MONTHS from the date of mailing of this Requirement for Information to correct the above noted deficiency (clarification regarding the existence of any assignee). Failure to respond completely to the Requirement will result in abandonment of the application. The time for response, however, may be extended by compliance with the provisions of 37 CFR 1.136(a).

Further correspondence with respect to this matter should be addressed as follows:

By mail: Commissioner of Patents and Trademarks
Box DAC
Washington, D.C. 20231

By FAX: (703) 308-6916
Attn: Special Program Law Office

By hand: One Crystal Park, Suite 520
2011 Crystal Drive
Arlington, VA

Telephone inquiries specific to this matter should be directed to the undersigned at (703) 305-9282.



Hiram H. Bernstein
Senior Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

#6

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. patent application of:

Mark H. SKOLNICK et al.

Serial No.: 08/289,221

Attn: Office of Deputy Assistant

Commissioner

Filed: 12 August 1994

RECEIVED

AUG 15 1995

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

OFFICE OF PETITIONS
AND PATENTS

RESPONSE TO REQUIREMENT FOR INFORMATION

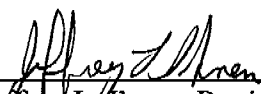
Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

In response to the Requirement for Information mailed 18 April 1995, Applicants note the existence of the following assignees: Myriad Genetics, Inc., the University of Utah Research Foundation and the United States of America, as represented by the Secretary of the Department of Health and Human Services. Transmitted herewith are Consents of Assignee for each assignee, Certificates Under 37 CFR §3.73(b) for each assignee and a Petition for Extension of Time for responding to the Requirement.

Consideration of the Petition filed 13 February 1995 is requested in view of this information.

Respectfully submitted


Jeffrey L. Ihnen, Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347-1
Dated: 13 August 1995
6565.01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

CONSENT OF ASSIGNEE

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

An Assignee, Myriad Genetics, Inc., of the above-identified application, assignment of which is being filed concurrently herewith, hereby consents to the amendment of inventorship to add Roger W. Wiseman and P. Andrew Futreal to the inventors named on the application filed on 12 August 1994 under 37 C.F.R. §1.53(d).

MYRIAD GENETICS, INC.

4/26/95
Date

By: Peter D. Meldrum
Peter D. Meldrum
President

CERTIFICATE UNDER 37 CFR §3.73(b)

Applicant: Mark H. SKOLNICK et al.

Application No.: 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

MYRIAD GENETICS, INC., a corporation, certifies that it is an assignee of the entire right, title and interest in the patent application identified above by virtue of:

A chain of title from the inventors of the patent application identified above, to the current assignee as shown below:

1. From: Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens
and Sean V. Tavtigian
To: Myriad Genetics, Inc.
for which a copy is attached.

Copies of assignments or other documents in the chain of title are attached.

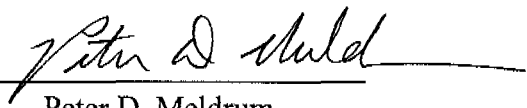
The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of his knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon.

For: MYRIAD GENETICS, INC.

Date: 4/26/95

Signature: 

Name:

Peter D. Meldrum

Title:

President

147071-1

ASSIGNMENT

WHEREAS, WE, Alexander KAMB, a citizen of the United States of America residing at 1103 East 600 South, Salt Lake City, Utah 84102; Keith D. HARSHMAN, a citizen of the United States of America residing at 159 North F Street, Salt Lake City, Utah 84103; Donna M. SHATTUCK-EIDENS, a citizen of the United States of America residing at 1874 Texas Street, Salt Lake City, Utah 84108; and Sean V. TAVTIGIAN, a citizen of the United States of America residing at 557 East 1st Ave, #3, Salt Lake City, Utah 84103 have jointly invented certain new and useful improvements in

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

for which an application for Letters Patent of the United States was filed on 12 August 1994 and assigned Serial No. 08/289,221, and

WHEREAS, MYRIAD GENETICS, INC., a corporation duly organized under the laws of the State of Utah, having a place of business at 417 Wakara Way, Suite 300, Salt Lake City, Utah 84108 (hereinafter referred to as "Assignee") is desirous of acquiring the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, including any and all continuations, divisions, reissues, or extensions, of such application or Letters Patent;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN, be it known that for good and valuable consideration, we have bargained, sold and assigned, transferred and set over and by these presents do hereby bargain, sell, assign, transfer and set over unto Assignee, its successors, assigns or other legal representatives, the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, and any and all continuations, divisions, reissues, or extensions of any such Letters Patents; the said to be held and enjoyed by Assignee for its own use and on behalf of its successors, assigns, or other legal representatives, to the full end of the term or terms for which said Letters Patents may be granted, reissued, or extended as fully and entirely as the same would have been held and enjoyed by us, jointly and severally, if this assignment and sale had not been made.

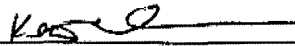
We further covenant and agree that we will at any time, upon request, execute and deliver any and all papers that may be necessary or desirable to perfect the title to said invention, or any Letters Patents that may be granted therefor, in Assignee, its successors, assigns, or other legal representatives, and that if Assignee, its successors, assigns or other legal representatives desire to secure a reissue or extension or division of any Letters Patents, we will upon request sign all papers, make all rightful oaths, and do all legal acts necessary and requisite for application of such reissue, extension or division and the procuring thereof, but at the expense of Assignee, its successors, assigns or other legal representatives.


We hereby further covenant and agree that we will at any time, upon request, communicate to Assignee, its successors, assigns or other legal representatives, any facts relating to the said invention and Letters Patents, or to the history thereof known to us, and will testify to the same in any interference or litigation, when requested to do so.


We hereby authorize and request the Commissioner of Patents to issue any and all Letters Patents covering said invention, or resulting from the aforesaid application to Assignee as the assignee of the entire right, title and interest in and to the said application and invention.

IN WITNESS WHEREOF, we have hereunto set our hand and seal on the date(s) given below.

Date 12/21/94 
Alexander KAMB

Date 12-17-94 
Keith D. HARSHMAN

Date 12/13/94 
Donna M. SHATTUCK-EIDENS

Date 12/13/94 
Sean V. TAVTIGIAN

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

CONSENT OF ASSIGNEE

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

An Assignee, the University of Utah Research Foundation, of the above-identified application, assignments of which are being filed concurrently herewith, hereby consents to the amendment of inventorship to add Roger W. Wiseman and P. Andrew Futreal to the inventors named on the application filed on 12 August 1994 under 37 C.F.R. §1.53(d).

UNIVERSITY OF UTAH RESEARCH FOUNDATION

4/28/95
Date

By: Thomas Major
Thomas Major
Director, Technology Transfer Office

CERTIFICATE UNDER 37 CFR §3.73(b)

Applicant: Mark H. SKOLNICK et al.

Application No.: 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

UNIVERSITY OF UTAH RESEARCH FOUNDATION, a university foundation, certifies that it is an assignee of the entire right, title and interest in the patent application identified above by virtue of:

A chain of title from the inventors of the patent application identified above, to the current assignee as shown below:

1. From: Mark H. Skolnick, David E. Goldgar, Yoshio Miki and Jeff Swenson
To: University of Utah
for which a copy is attached.
2. From: University of Utah
To: University of Utah Research Foundation
for which a copy is attached.

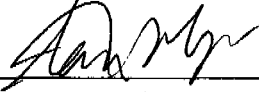
Copies of assignments or other documents in the chain of title are attached.

The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of his knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon.

For: UNIVERSITY OF UTAH RESEARCH FOUNDATION

Date: 4/28/95 Signature: 
Name: Tom Major
Title: Director, Technology Transfer Office

ASSIGNMENT

WHEREAS, WE, Mark H. SKOLNICK, a citizen of the United States of America residing at 460 Northmount Way, Salt Lake City, Utah 84103; David E. GOLDGAR, a citizen of the United States of America residing at 2707 East Kelly Lane, Salt Lake City, Utah 84117; Yoshio MIKI, a citizen of Japan residing at 40 South 900 East, Salt Lake City, Utah 84102; and Jeff SWENSON, a citizen of the United States of America residing at 745 West Creek Bend, #275, Salt Lake City, Utah 84117 have jointly invented certain new and useful improvements in

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

for which an application for Letters Patent of the United States was filed on 12 August 1994 and assigned Serial No. 08/289,221, and

WHEREAS, THE UNIVERSITY OF UTAH, a non-profit institution duly organized under the laws of the State of Utah, having a place of business at 421 Wakara Way, Suite 170, Salt Lake City, Utah 84108 (hereinafter referred to as "Assignee") is desirous of acquiring the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, including any and all continuations, divisions, reissues, or extensions, of such application or Letters Patent;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN, be it known that for good and valuable consideration, we have bargained, sold and assigned, transferred and set over and by these presents do hereby bargain, sell, assign, transfer and set over unto Assignee, its successors, assigns or other legal representatives, the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, and any and all continuations, divisions, reissues, or extensions of any such Letters Patents; the said to be held and enjoyed by Assignee for its own use and on behalf of its successors, assigns, or other legal representatives, to the full end of the term or terms for which said Letters Patents may be granted, reissued, or extended as fully and entirely as the same would have been held and enjoyed by us, jointly and severally, if this assignment and sale had not been made.

We further covenant and agree that we will at any time, upon request, execute and deliver any and all papers that may be necessary or desirable to perfect the title to said invention, or any Letters Patents that may be granted therefor, in Assignee, its successors, assigns, or other legal representatives, and that if Assignee, its successors, assigns or other legal representatives desire to secure a reissue or extension or division of any Letters Patents, we will upon request sign all papers, make all rightful oaths, and do all legal acts necessary and requisite for application of such reissue, extension or division and the procuring thereof, but at the expense of Assignee, its successors, assigns or other legal representatives.

We hereby further covenant and agree that we will at any time, upon request, communicate to Assignee, its successors, assigns or other legal representatives, any facts relating to the said invention and Letters Patents, or to the history thereof known to us, and will testify to the same in any interference or litigation, when requested to do so.

We hereby authorize and request the Commissioner of Patents to issue any and all Letters Patents covering said invention, or resulting from the aforesaid application to Assignee as the assignee of the entire right, title and interest in and to the said application and invention.

IN WITNESS WHEREOF, we have hereunto set our hand and seal on the date(s) given below.

Dec 13, 1994
Date

Mark Skolnick
Mark H. SKOLNICK

Dec 14 1994
Date

David E. Goldgar
David E. GOLDGAR

Dec 14 1994
Date

Yoshio Miki
Yoshio MIKI

Dec 14 1994
Date

Jeff Swenson
Jeff SWENSON

ASSIGNMENT

WHEREAS, THE UNIVERSITY OF UTAH, a non-profit institution duly organized under the laws of the State of Utah, having a place of business at 421 Wakara Way, Suite 170, Salt Lake City, Utah 84108 (hereinafter referred to as "Assignor") is the owner, by virtue of an assignment executed concurrently herewith by coinventors Mark H. SKOLNICK, David E. GOLDBAR, Yoshio MIKI and Jeff SWENSON of the following:

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

for which an application for Letters Patent of the United States was filed on 12 August 1994 and Serial No. 08/289,221, and

WHEREAS, THE UNIVERSITY OF UTAH RESEARCH FOUNDATION, a non-profit organization duly organized under the laws of the State of Utah, having a place of business at 421 Wakara Way, Suite 170, Salt Lake City, Utah 84108 (hereinafter referred to as "Assignee") is desirous of acquiring the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, including any and all continuations, divisions, reissues, or extensions, of such application or Letters Patent;

NOW, THEREFORE, TO ALL WHOM IT MAY CONCERN, be it known that for good and valuable consideration, Assignor has bargained, sold and assigned, transferred and set over and by these presents does hereby bargain, sell, assign, transfer and set over unto Assignee, its successors, assigns or other legal representatives, the entire right, title and interest in and to the aforesaid application in which the invention is disclosed, described and claimed, in and to the above-identified invention for the United States of America and all countries foreign thereto, and in and to any and all Letters Patents to be issued for said invention in the United States of America and all countries foreign thereto, and any and all continuations, divisions, reissues, or extensions of any such Letters Patents; the said to be held and enjoyed by Assignee for its own use and on behalf of its successors, assigns, or other legal representatives, to the full end of the term or terms for which said Letters Patents may be granted, reissued, or extended as fully and entirely as the same would have been held and enjoyed by us, jointly and severally, if this assignment and sale had not been made.

Assignor further covenants and agrees that Assignor will at any time, upon request, execute and deliver any and all papers that may be necessary or desirable to perfect the title to said invention, or any Letters Patents that may be granted therefor, in Assignee, its successors, assigns, or other legal representatives, and that if Assignee, its successors, assigns or other legal representatives desire to secure a reissue or extension or division of any Letters Patents, Assignor will upon request sign all papers, make all rightful oaths, and do all legal acts necessary and requisite for application of such reissue, extension or division and the procuring thereof, but at the expense of Assignee, its successors, assigns or other legal representatives.

Assignor hereby further covenants and agrees that Assignor will at any time, upon request, communicate to Assignee, its successors, assigns or other legal representatives, any facts relating to the said invention and Letters Patents, or to the history thereof known to us, and will testify to the same in any interference or litigation, when requested to do so.

Assignor hereby authorizes and requests the Commissioner of Patents to issue any and all Letters Patents covering said invention, or resulting from the aforesaid application to Assignee as the assignee of the entire right, title and interest in and to the said application and invention.

IN WITNESS WHEREOF, Assignor has hereunto set its hand and seal on the date(s) given below.

12/13/94
Date

THE UNIVERSITY OF UTAH

By:

Thomas D. Major
Thomas Major
Director
Technology Transfer Office

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

CONSENT OF ASSIGNEE

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

An Assignee, the United States of America, as represented by the Secretary of the Department of Health and Human Services, of the above-identified application, assignment of which is being filed concurrently herewith, hereby consents to the amendment of inventorship to add Roger W. Wiseman and P. Andrew Futreal to the inventors named on the application filed on 12 August 1994 under 37 C.F.R. §1.53(d).

UNITED STATES OF AMERICA, as represented by the
Secretary of the Department of Health and Human Services

8 August 1995
Date

By: Susan Rucker

Susan Rucker, Patent Advisor
for the Director, Dr. Marie Freire
Office of Technology Transfer

CERTIFICATE UNDER 37 CFR §3.73(b)

Applicant: Mark H. SKOLNICK et al.

Application No.: 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

The UNITED STATES OF AMERICA as represented by the Secretary of the Department of Health and Human Services, certifies that it is an assignee of the entire right, title and interest in the patent application identified above by virtue of:

A chain of title from the inventors of the patent application identified above, to the current assignee as shown below:

1. From: Roger W. Wiseman and P. Andrew Futreal
To: the United States of America as represented by the Secretary of the Department of Health and Human Services
for which a copy is attached.

Copies of assignments or other documents in the chain of title are attached.

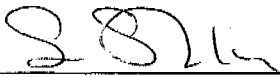
The undersigned has reviewed all the documents in the chain of title of the patent application identified above and, to the best of her knowledge and belief, title is in the assignee identified above.

The undersigned (whose title is supplied below) is empowered to act on behalf of the assignee.

I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon.

For: The UNITED STATES OF AMERICA as represented by
the Secretary of the Department of Health and Human Services

Date: 8/8/95

Signature: 

Name:

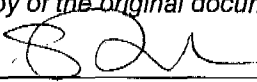
Susan Rucker

Title:

Patent Advisor

for the Director, Dr. Marie Freire
Office of Technology Transfer

RECORDATION FORM COVER SHEET
PATENTS ONLY

1. Name of Conveying party(ies): P. Andrew Futreal Roger W. Wiseman		2. Name and address of receiving party(ies): The Government of the United States of America, as represented by the Secretary, Department of Health and Human Services Internal Address: Office of Technology Transfer National Institutes of Health 6011 Executive Blvd. Rockville, MD 20852-3804 Street Address: same as above	
3. Nature of conveyance: [X] Assignment Execution Date: August 1, 1995 and July 31, 1995			
4. Application number(s) or patent numbers: A. Patent Application No.(s): Serial Number 08/289,221 filed August 12, 1994 entitled: 17q-Linked Breast and Ovarian Cancer Susceptibility Gene			
5. Name and address of party to whom correspondence concerning document should be mailed: Name: <u>Susan S. Rucker</u> Internal Address: Office of Technology Transfer National Institutes of Health 6011 Executive Blvd. Rockville, MD 20852-3804 Street Address: same as above		6. Total Number of patents and applications involved: <u>1</u>	
		7. Total Fee (37 C.F.R. §3.41) \$80.00 [X] Please charge any deficiency or credit any overpayment to the below-numbered deposit account.	
		8. Deposit Account Number: <u>14-1052</u>	
9. Statement and signature. <i>To the best of my knowledge and belief, the foregoing information is true and correct and any attached copy is a true copy of the original document.</i> <u>Susan S. Rucker (35,762)</u>  <u>August 3, 1995</u> Name of Person Signing Signature Date			
Total Number of pages comprising cover sheet <u>1</u>			

ASSIGNMENT

WHEREAS, we, Roger W. Wiseman and P. Andrew Futreal, employees of the Department of Health and Human Services and Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens, and Sean V. Tavtigian, have invented "17q-Linked Breast and Ovarian Cancer Susceptibility Gene" for which we have made application for Letters Patent of the United States Serial No. 08/289,221 filed August 12, 1994; and

WHEREAS, we are the applicants named in the above-identified application for Letters Patent; and

WHEREAS, the conditions under which said invention was made are such as to entitle THE UNITED STATES OF AMERICA, as represented by the Secretary, Department of Health and Human Services [hereinafter THE GOVERNMENT] under Paragraph 1(a) of Executive Order 10096 to the entire right, title, and interest herein, both domestic and foreign; and

WHEREAS, THE GOVERNMENT is desirous of acquiring all domestic and foreign right, title, and interest in the above-mentioned invention described in the application for Letters Patent; and

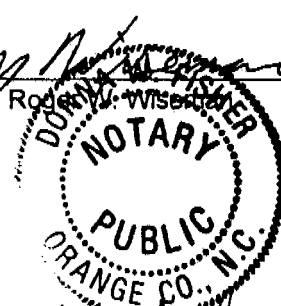
NOW, THEREFORE, for good and valuable consideration, the receipt of which is hereby acknowledged, we hereby assign and transfer to THE GOVERNMENT, represented by the Secretary, Department of Health and Human Services, the full and exclusive rights in and to said invention in the United States and within each and every foreign country in which THE GOVERNMENT elects to file and the entire right, title, and interest in and to such applications, and any continuations, continuations-in-part, divisions, reissues or extensions thereof, and including priority rights as may be filed in the United States and foreign countries, and such Letters Patent as may be granted to be held by THE GOVERNMENT to the end of the term for which the same would have been held by the inventors had this assignment not been made.

We further agree to make, execute, and deliver to the Secretary, Department of Health and Human Services, upon request, any and all papers, documents, affidavits, or other instruments that may be necessary in the prosecution of any application or applications for improvements or reissues of Letters Patent, and to assist THE GOVERNMENT in every way as may be requested in protecting said invention, provided that any expense of extending such assistance shall be paid by THE GOVERNMENT.

IN WITNESS WHEREOF we subscribe our hands and seals on the dates shown below:

7/31/95
Date
County of Durham
State of North Carolina
Ss:
Subscribed and sworn to before me this 31st day of July 1995.

[Seal]


Donna W. Fisher
Notary Public

ASSIGNMENT
Attorney Docket No.: NIH E-237-94/2
UU U-2090

Date P. Andrew Futreal

County of _____
Ss:

State of _____

Subscribed and sworn to before me this ____ day of _____, 1995.

[Seal]

Notary Public

ASSIGNMENT

WHEREAS, we, **Roger W. Wiseman** and **P. Andrew Futreal**, employees of the Department of Health and Human Services and **Mark H. Skolnick**, **David E. Goldgar**, **Yoshio Miki**, **Jeff Swenson**, **Alexander Kamb**, **Keith D. Harshman**, **Donna M. Shattuck-Eidens**, and **Sean V. Tavtigian**, have invented "**17q-Linked Breast and Ovarian Cancer Susceptibility Gene**" for which we have made application for Letters Patent of the United States Serial No. **08/289,221** filed **August 12, 1994**; and

WHEREAS, we are the applicants named in the above-identified application for Letters Patent; and

WHEREAS, the conditions under which said invention was made are such as to entitle **THE UNITED STATES OF AMERICA**, as represented by the Secretary, Department of Health and Human Services [hereinafter **THE GOVERNMENT**] under Paragraph 1(a) of Executive Order 10096 to the entire right, title, and interest herein, both domestic and foreign; and

WHEREAS, **THE GOVERNMENT** is desirous of acquiring all domestic and foreign right, title, and interest in the above-mentioned invention described in the application for Letters Patent; and

NOW, THEREFORE, for good and valuable consideration, the receipt of which is hereby acknowledged, we hereby assign and transfer to **THE GOVERNMENT**, represented by the Secretary, Department of Health and Human Services, the full and exclusive rights in and to said invention in the United States and within each and every foreign country in which **THE GOVERNMENT** elects to file and the entire right, title, and interest in and to such applications, and any continuations, continuations-in-part, divisions, reissues or extensions thereof, and including priority rights as may be filed in the United States and foreign countries, and such Letters Patent as may be granted to be held by **THE GOVERNMENT** to the end of the term for which the same would have been held by the inventors had this assignment not been made.

We further agree to make, execute, and deliver to the Secretary, Department of Health and Human Services, upon request, any and all papers, documents, affidavits, or other instruments that may be necessary in the prosecution of any application or applications for improvements or reissues of Letters Patent, and to assist **THE GOVERNMENT** in every way as may be requested in protecting said invention, provided that any expense of extending such assistance shall be paid by **THE GOVERNMENT**.

IN WITNESS WHEREOF we subscribe our hands and seals on the dates shown below:

Date Roger W. Wiseman

County of _____
Ss:

State of _____

Subscribed and sworn to before me this ____ day of _____, 1995.

[Seal]

Notary Public

ASSIGNMENT
Attorney Docket No.: NIH E-237-94/2
UU U-2090

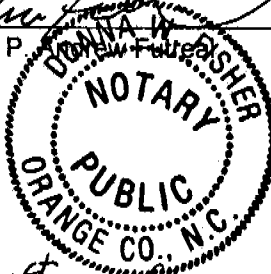
8/1/95
Date

Donna W. Fisher
P

County of Durham

Ss:

State of North Carolina



Subscribed and sworn to before me this 1st day of August, 1995.

[Seal]

Donna W. Fisher
Notary Public



#17 0360 10-17-95
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. patent application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

RECEIVED
NOV 01 1995
OFFICE OF PATENTS
& TRADEMARKS

Attn: Office of Deputy Assistant
Commissioner

SUPPLEMENTAL RESPONSE TO REQUIREMENT FOR INFORMATION

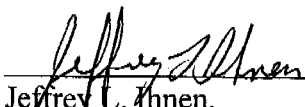
Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Further to the Response to Requirement for Information filed on 28 August 1995, Applicants submit herewith a Verified Statement of Facts and a Declaration and Power of Attorney which have been executed by David E. Goldgar.

Consideration of the Petition filed 13 February 1995 is requested in view of this additional information.

Respectfully submitted,


Jeffrey L. Ahnen,
Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI, LLP
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810
Atty. Docket No. 24884-109347-1
Dated: 26 October 1995
647.01

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. Application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE



VERIFIED STATEMENT OF FACTS

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Mark H. Skolnick, David E. Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith D. Harshman, Donna M. Shattuck-Eidens and Sean V. Tavtigian declare that:

(1) The invention described and claimed in the above-identified application was developed in part by us and in part by Roger W. Wiseman and P. Andrew Futreal.

(2) Mark Skolnick, David Goldgar, Yoshio Miki and Jeff Swenson are employees of the University of Utah (University) and have been conducting research to identify and characterize a breast cancer gene on human chromosome 17 since at least January 1991. This research has led to three earlier-filed related applications of which Skolnick, Goldgar, Miki and Swenson are coinventors.

(3) Mark Skolnick and his fellow University researchers began collaborating with Myriad Genetics, Inc. (Myriad) and its researchers on May 1, 1992 to intensify the search for the breast cancer gene. As a result of this collaborative effort, Mark Skolnick, David Goldgar, Yoshio Miki, Jeff Swenson, Alexander Kamb, Keith Harshman and Donna Shattuck-Eidens identified a gene which was disclosed and claimed in a fourth, earlier-filed

related application as a breast cancer gene. This fourth related application also disclosed other candidate DNA sequences.

(4) Mark Skolnick and his fellow University researchers began collaborating with Roger Wiseman and his fellow researchers at the National Institute of Environmental Health Sciences (NIEHS) in April 1992 to intensify the search for the breast cancer gene. The researchers at Myriad were included in this collaboration, as well as researchers at other universities and companies.

(5) Mark Skolnick, Alexander Kamb or Donna Shattuck-Eidens periodically communicated research results with Jeffrey Ihnen, patent counsel to the University and Myriad, to update him on the results of their research. In early August 1994, Jeffrey Ihnen was advised that the gene identified in the fourth related application as being the breast cancer gene was not thought to be the correct gene and that about 70% of the DNA coding sequence for the correct gene had been determined. During a meeting on 10 August 1994 with Jeffrey Ihnen, it was decided to proceed with the filing of an application to claim the gene sequence which was then known as well as the predisposing mutations which had been discovered. It was also decided that the application would be filed on 12 August 1994.

(6) Inventorship of the breast cancer gene was briefly discussed during the 10 August 1994 meeting. Based on the available information, it was determined that the inventorship for the breast cancer gene was as set forth in the transmittal letter for this application. That is, it was determined that Roger Wiseman and Andrew Futreal were not joint inventors of the breast cancer gene, since they did not develop the entire gene sequence (then known) and had not discovered any mutations in the gene.

(7) The application was filed on 12 August 1994 without a Declaration, pursuant to the provisions of 37 CFR §1.53(d), naming us as joint inventors.

(8) On 5 October 1994, following the filing of the present application, NIEHS claimed that Wiseman and Futreal were joint inventors of the application, since claim 3 in the application was directed to fragments of the breast cancer gene. On 28 November 1994 NIEHS

provided information from its research records describing the identification of fragments of coding sequences by Wiseman and Futreal from genetic materials obtained from Myriad. After a thorough analysis was made of all of the pertinent research records of the University and Myriad and of the information provided by NIEHS, it was determined that Wiseman and Futreal had been involved in the identification of two DNA fragments which are part of the breast cancer gene. After the facts were completely analyzed, it was recognized that Wiseman and Futreal are joint inventors as to a fragment claim and had been inadvertently omitted, without any deceptive intent, from the application as filed under 37 CFR §1.53(d) and should be properly joined as coinventors of the claimed subject matter.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

October 23 1995
Date

Mark H. Skolnick

David E. Goldgar
David E. Goldgar

Date

Yoshio Miki

Date

Jeff Swenson

Date

Alexander Kamb

Date

Keith D. Harshman

Date

Donna M. Shattuck-Eidens

Date

Sean V. Tavtigian

g:\pat\myriad\pto\papers\brca1\221ver.stm

DECLARATION AND POWER OF ATTORNEY

As below-named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

17q-LINKED BREAST AND OVARIAN CANCER SUSCEPTIBILITY GENE

and which was filed on 12 August 1994 and assigned Serial No. 08/289,221.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above.

I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) on which priority is claimed:

<u>Prior Foreign Application(s)</u>			<u>Priority Claimed</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>
<u>Appln. No.</u>	<u>Country</u>	<u>Date Filed</u>	<u>Yes No</u>

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose all information known to be material to patentability as defined in Title 37, Code of Federal Regulations, § 1.56 which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

<u>08/249,602</u> (Appln. No.)	<u>20 May 1994</u> (Filing Date)	<u>Pending</u> (Status: patented, pending, abandoned)
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>
<u>(Appln. No.)</u>	<u>(Filing Date)</u>	<u>(Status: patented, pending, abandoned)</u>

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both,

under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I hereby appoint Jeffrey L. Ihnen, Registration No. 28,957; Fred W. Hathaway, Registration No. 32,236; Royal W. Craig, Registration No. 34,145; and E. Brendan Magrab, Registration No. 36,205; all of Venable, Baetjer, Howard & Civiletti, whose address is 1201 New York Avenue, N.W., Suite 1000, Washington, D.C. 20005, telephone number (202) 962-4800, my attorneys and/or agents with full power of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Please direct all correspondence and telephone calls to Venable, Baetjer, Howard & Civiletti at the above address and telephone number.

Full Name of Sole or First Inventor	<u>Mark H. SKOLNICK</u>
Inventor's Signature	_____
Date of Signature	_____
Residence Address	<u>460 Northmount Way</u> <u>Salt Lake City, Utah 84103</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>USA</u>
Full Name of Second Inventor	<u>David E. GOLDGAR</u>
Inventor's Signature	<u>David E. Goldgar</u>
Date of Signature	<u>October 23 1995</u>
Residence Address	<u>2707 East Kelly Lane</u> <u>Salt Lake City, Utah 84117</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>USA</u>
Full Name of Third Inventor	<u>Yoshio MIKI</u>
Inventor's Signature	_____
Date of Signature	_____
Residence Address	<u>40 South 900 East</u> <u>Salt Lake City, Utah 84102</u>
Post Office Address	<u>SAME</u>
Country of Citizenship	<u>JAPAN</u>

Full Name of Fourth
Inventor

Jeff SWENSON

Inventor's Signature

Date of Signature

Residence Address

745 West Creek Bend. #2807
Salt Lake City, Utah 84117

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Fifth
Inventor

Alexander KAMB

Inventor's Signature

Date of Signature

Residence Address

1103 East 600 South
Salt Lake City, Utah 84102

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Sixth
Inventor

Keith D. HARSHMAN

Inventor's Signature

Date of Signature

Residence Address

159 North F Street
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Seventh
Inventor

Donna M. SHATTUCK-EIDENS

Inventor's Signature

Date of Signature

Residence Address

1874 Texas Street
Salt Lake City, Utah 84108

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Eighth
Inventor

Sean V. TAVTIGIAN

Inventor's Signature

Date of Signature

Residence Address

557 East 1st Avenue, #3
Salt Lake City, Utah 84103

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Ninth
Inventor

Roger W. WISEMAN

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA

Full Name of Tenth
Inventor

P. Andrew FUTREAL

Inventor's Signature

Date of Signature

Residence Address

Post Office Address

SAME

Country of Citizenship

USA



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
ASSISTANT SECRETARY AND COMMISSIONER
OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

Paper No. 8

Venable, Baetjer, Howard & Civiletti
1201 New York Avenue, N.W.
Suite 1000
Washington, D.C. 20005

COPY MAILED

MAR 11 1996

**OFFICE OF PETITIONS
AND PATENTS**

In re Application of	:	
Mark H. Skolnick, et al	:	
Application No. 08/289,221	:	DECISION ON PETITION UNDER
Filed: August 12, 1994	:	37 CFR 1.48(a)
For: 17q-LINKED BREAST AND	:	
OVARIAN CANCER	:	
SUSCEPTABILITY GENE	:	

This is a decision on a February 13, 1995 petition under 37 CFR 1.48(a), requesting correction of the inventorship and on the papers of August 15, 1995 and October 26, 1995 filed in support thereof, which August 15, 1995 papers timely respond (with a petition and fee under 37 CFR 1.136(a) for a two month extension of time) to an April 18, 1995 Requirement for Information.

The §1.48(a) petition to correct inventorship is Granted.

The Requirement for Information required written consents and § 3.73(b) certifications from three assignees, which were submitted on August 15, 1995. An October 26, 1995 paper has submitted a duplicate § 1.63 declaration and a duplicate verified statement of facts from original named inventor Goldgar over what was submitted on February 13, 1995.

Based on the file record as a whole and the facts as set forth in the Requirement for Information and in the statements now of record, the error in inventorship occurred without deceptive intent and was diligently corrected.

The \$370 § 1.136(a) petition fee for a two month extension of time will be charged to Deposit Account No. 22-0261 as authorized in the August 15, 1995 Response.

The application is being returned to the Initial Patent Examination Division of the Office of Initial Patent Examination for further processing, including issuance of a filing receipt,

Application No. 08 89,221

Page 2

with the names of the inventors as shown on the executed
declarations under 37 CFR 1.63 filed on February 13, 1995.



Hiram H. Bernstein
Senior Legal Advisor
Special Program Law Office
Office of the Deputy Assistant Commissioner
for Patent Policy and Projects

(703) 305-9285

SAC

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. patent application of:

Mark H. SKOLNICK et al.

Serial No.: 08/289,221

Attn: Office of Deputy Assistant
Commissioner

Filed: 12 August 1994

For: 17q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

RECEIVED

MAR 12 1996

OFFICE OF PETITIONS
AND PATENTS

PETITION FOR EXTENSION OF TIME

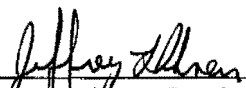
Hon. Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Petition is hereby made for an extension of two (2) months in which to respond to the Requirement for Information mailed 18 April 1995.

Please charge the requisite petition fee of \$370.00 to Deposit Account 22-0261. This Petition is submitted in triplicate. If any discrepancies in fees are noted, the same should be charged to Deposit Account 22-0261.

Respectfully submitted


Jeffrey L. Ilmen, Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347-1
Dated: 13 August 1995
6565.01

AA11062 03/13/96 08289221

22-0261 110 116

370.00CH

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. patent application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Examiner:

Filed: 12 August 1994

Group Art Unit: 1804

For: 17Q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

#2098
2098
2098
2098


EXPRESS ABANDONMENT

Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicants hereby expressly abandon the above-identified application, as of the date
given hereinbelow.

Respectfully submitted


Jeffrey L. Ihnen
Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347
Dated: 09 September 1996

** TOTAL PAGE.003 **

SEP 11 '96 12:32 FR VENABLE BOETJER

202 962 8300 TO 47#17033057401# P.02/03



(PTO Receipt Stamp Here)

Receipt for Documents Filed

IN RE. ☒ PATENT ☐ TM

INVENTOR/APPLICANT/OWNER: Mark H. SKOLNICK et al.

SERIAL NO. 08/289,221

REG. NO.

COUNTRY:

PAT. NO.

APPLICATION FILING DATE 12 August 1994

FOR: ☐ MARK ☒ TITLE 17q-LINKED BREAST AND OVARIAN CANCER
SUSCEPTIBILITY GENE

DOCUMENTS FILED:

=====

EXPRESS ABANDONMENT

=====

DATE FILED: 10 September 1996

CLIENT/
MATTER NO. 24884-109347

DOB DATE: (NONE)

CLIENT NAME: Myriad

ATTY/SEC. JLI:ch

Venable, Baetjer, Howard & Civiletti, LLP - 1201 New York Avenue, N.W., Suite 1000, - Washington, D.C. 20005
Telephone No. (202) 962-4800 - Facsimile: (202) 962-8300
10407.01 DC2DOCS

SEP 11 '96 12:32 FR VENABLE BAETJER

202 962 8300 TO 47#17033057401# P.01/03

VENABLE, BAETJER, HOWARD & CIVILETTI, LLP
1201 New York Avenue, N.W., Suite 1000
Washington, D.C. 20005
Telephone No. (202) 962-4800 Telecopy No. (202) 962-8300

FACSIMILE COVER SHEET

DATE: 11 September 1996
SEND: IMMEDIATELY

OUR FILE NO.: 24884-109347
USER ID NO.: 2260

TO: **Examiner Dianne Rees, Group Art Unit 1807**
U.S. Patent and Trademark Office

FACSIMILE NO.: (703) 305-7401

TELEPHONE NO.: (703) 308-6565

FROM: Jeffrey L. Ihnen

TELEPHONE NO.: (202) 962-4810

TOTAL NO. OF PAGES: two (excluding cover sheet)

MESSAGE: Re. U.S. Serial No. 08/289,221; Skolnick et al
Our Ref.: 24884-109347

SEE ATTACHED EXPRESS ABANDONMENT AND FILING RECEIPT

OFFICIAL
**FAX CENTER
RECEIVED**

SEP 11 1996

GROUP 1800

CONFIDENTIALITY NOTICE

"WARNING: UNAUTHORIZED INTERCEPTION OF THIS TELEPHONIC COMMUNICATION COULD BE A VIOLATION OF FEDERAL AND STATE LAW." The documents accompanying this facsimile transmission contain confidential information belonging to the sender which is legally privileged. The information is intended only for the use of the individual or entity named above. If you are not the intended recipient, you are hereby notified that any disclosure, copying, distribution or the taking of any action in reliance on the contents of this facsimiled information is strictly prohibited. If you have received this facsimile in error, please immediately notify us by telephone to arrange for return of the original documents to us.

If there are any problems with the transmission, please call as soon as possible (202) 962-4994. Thank you!



UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/289,221	08/12/94	SKOLNICK	M 248841093471

VENABLE BAETJER HOWARD & CIVILETTI
1201 NEW YORK AVENUE NW
SUITE 1000
WASHINGTON DC 20005

18M2/0919 7

REES, D	
EXAMINER	
ART UNIT	PAPER NUMBER
1807	10

DATE MAILED: 09/19/96

NOTICE OF ABANDONMENT

This application is abandoned in view of:

- ☐ Applicant's failure to respond to the Office letter, mailed _____.
- ☒ Applicant's letter of express abandonment which is in compliance with 37 C.F.R. 1.138.
- ☐ Applicant's failure to timely file the response received _____ within the period set in the Office letter.
- ☐ Applicant's failure to pay the required issue fee within the statutory period of 3 months from the mailing date of _____ of the Notice of Allowance.
 - ☐ The issue fee was received on _____.
 - ☐ The issue fee has not been received in Allowed Files Branch as of _____.

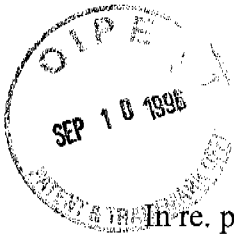
In accordance with 35 U.S.C. 151, and under the provisions of 37 C.F.R. 1.316(b), applicant(s) may petition the Commissioner to accept the delayed payment of the issue fee if the delay in payment was unavoidable. The petition must be accompanied by the issue fee, unless it has been previously submitted, in the amount specified by 37 C.F.R. 1.17(l), and a verified showing as to the causes of the delay.

If applicant(s) never received the Notice of Allowance, a petition for a new Notice of Allowance and withdrawal of the holding of abandonment may be appropriate in view of *Delgar Inc. v. Schuyler*, 172 U.S.P.Q. 513.

- ☐ Applicant's failure to timely correct the drawings and/or submit new or substitute formal drawings by _____ as required in the last Office action.
 - ☐ The corrected and/or substitute drawings were received on _____.
- ☐ The reason(s) below.

W. GARY JONES
SUPERVISORY PATENT EXAMINER
GROUP 1800

9/14/96



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re. patent application of:

Mark H. SKOLNICK et al.

Serial No. 08/289,221

Examiner:

Filed: 12 August 1994

Group Art Unit: 1804

For: 17Q-LINKED BREAST AND OVARIAN
CANCER SUSCEPTIBILITY GENE

11
10/15/96
Q errors

EXPRESS ABANDONMENT

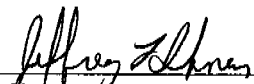
Hon. Commissioner of Patents
and Trademarks
Washington, D.C. 20231

Sir:

Applicants hereby expressly abandon the above-identified application, as of the date
given hereinbelow.

26 SEP 16 AM 11:00
GROUP 180

Respectfully submitted



Jeffrey L. Ihnen
Registration No. 28,957

VENABLE, BAETJER, HOWARD & CIVILETTI
1201 New York Avenue, N.W., #1000
Washington, D.C. 20005
(202) 962-4810

Atty. Docket No. 24884-109347
Dated: 09 September 1996

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

In re Application of

Shattuck-Eidenes

Application Number

Filed

08/289221

8-12-94

Group Art Unit

Examiner

Paper No. *12*

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

___ (A) referred to in United States Patent Number *5643473*, column ___.

___ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. _____, filed _____, on page _____ of paper number _____.

___ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. _____, filed _____, or

___ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

Calvin Perry

Signature

Calvin Perry

Typed or printed name

12-8-97

Date

PROCESSED BY
DEC 8 1997

FOR PTO USE ONLY

Approved by: *[Signature]*

(initials)

Unit: _____

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

PROCESSED BY
JAN 8 1999
FII

In re Application of <i>Shattuck - Eideus et al.</i>	
Application Number <i>S.W. 289,221</i>	Filed <i>8/12/94</i>
Group Art Unit	Examiner

Paper No. *#13*

Assistant Commissioner for Patents
 Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

- ☒ (A) referred to in United States Patent Number *5,693,473*, column _____
- ☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. _____, filed _____, on page _____ of paper number _____
- ☐ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. _____, filed _____, or
- ☐ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

Sarla C. Shroff

 Signature
SARLA C. SHROFF

 Typed or printed name

12/23/97

 Date

FOR PTO USE ONLY	
Approved by: <i>TS</i>	(initials)
Unit: <i>FII</i>	

REQUEST FOR ACCESS OF ABANDONED APPLICATION UNDER 37 CFR 1.14(a)

In re Application of

Application Number

08/289221

Filed

Jun 7, 1995

Group Art Unit

Examiner

DONNA M. SHATTECK-EIDERS.

Paper No. 14

Assistant Commissioner for Patents
Washington, DC 20231

I hereby request access under 37 CFR 1.14(a)(3)(iv) to the application file record of the above-identified ABANDONED application, which is: (CHECK ONE)

- ☒ (A) referred to in United States Patent Number 5693473, column _____
- ☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11, i.e., Application No. _____, filed _____, on page _____ of paper number _____
- ☐ (C) an application that claims the benefit of the filing date of an application that is open to public inspection, i.e., Application No. _____, filed _____, or
- ☐ (D) an application in which the applicant has filed an authorization to lay open the complete application to the public.

Please direct any correspondence concerning this request to the following address:

TRUNG THAI BERGER & ALTMAN 1/20/98

Signature

Date

TRUNG THAI BERGER & ALTMAN

Typed or printed name

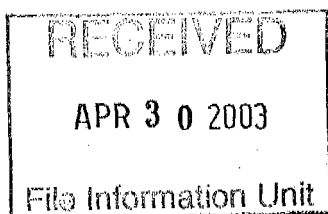
PROCESSED BY
FOR PTO USE ONLY 1998
JAN 20 1998

Approved by: FHH

Unit: _____

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

REQUEST FOR ACCESS TO AN APPLICATION UNDER 37 CFR 1.14(e)

In re Application of

Application Number

08/289221

Filed

9-12-94

Art Unit

Examiner

Paper No. 15Assistant Commissioner for Patents
Washington, DC 20231

1. ☒ I hereby request access under 37 CFR 1.14(e)(2) to the application file record of the above-identified ABANDONED Application, which is not within the file jacket of a pending Continued Prosecution Application (CPA) (37 CFR 1.53(d)) and is: (CHECK ONE)

☒ (A) referred to in:

United States Patent Application Publication No. _____, page _____, line _____.

United States Patent Number 5695473, column _____, line _____, or

an International Application which was filed on or after November 29, 2000 and which

designates the United States, WIPO Pub. No. _____, page _____, line _____.

☐ (B) referred to in an application that is open to public inspection as set forth in 37 CFR 1.11(b) or

1.14(e)(2)(i), i.e., Application No. _____, paper No. _____, page _____, line _____.

2. ☐ I hereby request access under 37 CFR 1.14(e)(1) to an application in which the applicant has filed an authorization to lay open the complete application to the public.

Signature

Date

Henry Dub

Typed or printed name

FOR PTO USE ONLY

Approved by: _____

(initials)

Unit: _____

Burden Hour Statement: This form is estimated to take 0.2 hours to complete. Time will vary depending upon the needs of the individual case. Any comments on the amount of time you are required to complete this form should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, Washington, DC 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Assistant Commissioner for Patents, Washington, DC 20231.

PATENT APPLICATION FEE DETERMINATION RECORD

Effective October 1, 1992

Application or Docket Number

08/289,221

CLAIMS AS FILED - PART I

SMALL ENTITY

OR

OTHER THAN
SMALL ENTITY

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA
BASIC FEE		
TOTAL CLAIMS	71 minus 20 = *	51
INDEPENDENT CLAIMS	19 minus 3 = *	16
MULTIPLE DEPENDENT CLAIM PRESENT		

RATE	FEE
	\$355.00
x\$11=	
x 37=	
+115=	
TOTAL	

RATE	FEE
	\$710.00
x\$22=	1122.00
x 74=	1184.00
+230=	
TOTAL	3016.00

* If the difference in column 1 is less than zero, enter "0" in column 2

CLAIMS AS AMENDED - PART II

SMALL ENTITY

OR

OTHER THAN
SMALL ENTITY

AMENDMENT A	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus **	=
	Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE
x\$11=	
x 37=	
+ 115=	
TOTAL	

RATE	ADDITIONAL FEE
x\$22=	
x 74=	
+230=	
TOTAL	

AMENDMENT B	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus **	=
	Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE
x\$11=	
x 37=	
+ 115=	
TOTAL	

RATE	ADDITIONAL FEE
x\$22=	
x 74=	
+ 230=	
TOTAL	

AMENDMENT C	(Column 1)	(Column 2)	(Column 3)	
	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA
	Total	*	Minus **	=
	Independent	*	Minus ***	=
FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM				

RATE	ADDITIONAL FEE
x\$11=	
x 37=	
+115=	
TOTAL	

RATE	ADDITIONAL FEE
x\$22=	
x 74=	
+230=	
TOTAL	

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.

** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".

*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".

The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.



MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET (FOR USE WITH FORM PTO-875)	SERIAL NO. <u>08/287,221</u>	FILING DATE
	APPLICANT(S)	

CLAIMS													
	AS FILED		AFTER 1st AMENDMENT		AFTER 2nd AMENDMENT			*		*		*	
	IND.	DEP.	IND.	DEP.	IND.	DEP.		IND.	DEP.	IND.	DEP.	IND.	DEP.
1	1						51	1	1				
2		1					52		1				
3		1					53		1				
4		1					54		1				
5	1						55	1					
6		1					56		1				
7	1						57		1				
8	1						58		1				
9	1						59		1				
10		1					60		1				
11		1					61		1				
12		1					62	1					
13		1					63	1					
14	1						64		1				
15	1						65		1				
16	1						66	1					
17		1					67	1					
18	1						68	1					
19		1					69	1					
20		1					70		1				
21		1					71		1				
22		1					72						
23		1					73						
24		1					74						
25		1					75						
26		1					76						
27		1					77						
28		1					78						
29		1					79						
30		1					80						
31		1					81						
32		1					82						
33		1					83						
34		1					84						
35		1					85						
36		1					86						
37		1					87						
38		1					88						
39		1					89						
40		1					90						
41	1						91						
42		1					92						
43		1					93						
44		1					94						
45	1						95						
46		1					96						
47		1					97						
48		1					98						
49		1					99						
50		1					100						
TOTAL IND.	11						TOTAL IND.	8					
TOTAL DEP.	39						TOTAL DEP.	13					
TOTAL CLAIMS							TOTAL CLAIMS						

PACE DATA ENTRY CODING SHEET

1ST EXAMINER

K. Mclean

DATE

8/23/94

2ND EXAMINER

Greene

DATE

7/9/96

APPLICATION NUMBER

08/289221

TYPE
APPL

1

FILING DATE

MONTH DAY YEAR

08/29/94

SPECIAL
HANDLING

0

GROUP
ART UNIT

1804

CLASS

435

SHEETS OF
DRAWING

004

TOTAL
CLAIMS

071

INDEPENDENT
CLAIMS

019

SMALL
ENTITY?

0

FILING FEE

3198

FOREIGN
LICENSE

4

ATTORNEY DOCKET NUMBER

248841093471

CONTINUITY DATA

CONT STATUS
CODE CODE

03 2

PARENT APPLICATION
SERIAL NUMBER

08246602

PCT APPLICATION SERIAL NUMBER

P	C	T	/					/							
P	C	T	/					/							
P	C	T	/					/							
P	C	T	/					/							
P	C	T	/					/							

PARENT PATENT
NUMBER

PARENT FILING
DATE

MONTH DAY YEAR

052094

PCT/FOREIGN APPLICATION DATA

FOREIGN
PRIORITY
CLAIMED

COUNTRY
CODE

PCT/FOREIGN APPLICATION SERIAL NUMBER

FOREIGN
FILING DATE

MONTH DAY YEAR
