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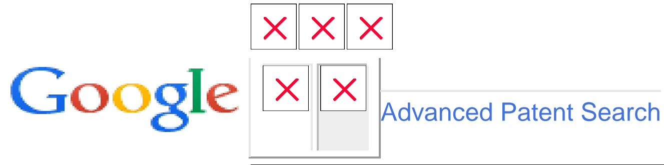

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Original Assignee	Myriad Genetics, Inc., Endo Recherche, Inc., Hsc Research & Development Limited Partnershi
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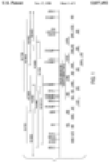
Chromosome 13-linked breast cancer susceptibility gene

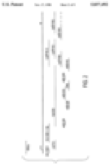
US 5837492 A

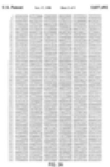
ABSTRACT

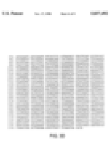
The present invention relates generally to the field of human genetics. Specifically, the present invention relates to method mutations in the BRCA2 gene and their use in the diagnosis of predisposition to breast cancer. The present invention furth cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to the therapy of huma the screening of the BRCA2 gene for mutations, which are useful for diagnosing the predisposition to breast cancer.

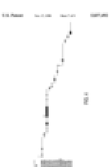
IMAGES(9)











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CLAIMS(30)

What is claimed is:

1. An isolated DNA molecule coding for a BRCA2 polypeptide, said DNA molecule comprising a nucleic acid sequence enc

2. The isolated DNA molecule of claim 1, wherein said DNA molecule comprises the nucleotide sequence set fo

3. The isolated DNA molecule of claim 1, wherein said DNA molecule is an allelic variant of the nucleotide sequ

4. The isolated DNA molecule of claim 1, which contains BRCA2 regulatory sequences.

5. An isolated DNA molecule comprising at least 15 contiguous nucleotides of the DNA molecule of claim 1.

6. An isolated DNA molecule coding for a mutated form of the BRCA2 polypeptide set forth in SEQ ID NO:2, wherein said r

7. The isolated DNA molecule of claim 6, wherein the DNA molecule comprises a mutated nucleotide sequence

8. The isolated DNA molecule of claim 7, wherein the mutation is selected from the group consisting of a deletio

9. An isolated DNA molecule comprising at least 15 contiguous nucleotides of the DNA of claim 6.

10. The isolated DNA molecule of claim 6 selected from the group consisting of:

(a) SEQ ID NO:1 having AC at nucleotide positions 277 and 278 deleted;

(b) SEQ ID NO:1 having four nucleotides at positions 982-985 deleted;

(c) SEQ ID NO:1 having four nucleotides at positions 4706-4709 deleted;

(d) SEQ ID NO:1 having C at nucleotide position 8525 deleted;

(e) SEQ ID NO:1 having five nucleotides at positions 9254-9258 deleted;

(f) SEQ ID NO:1 having GT at nucleotide positions 4075 and 4076 deleted;

(g) SEQ ID NO:1 having five nucleotides at positions 999-1003 deleted;

(h) SEQ ID NO:1 having T at nucleotide position 6174 deleted;

(i) SEQ ID NO:1 having three nucleotides at positions 4132-4134 deleted;

(j) SEQ ID NO:1 having a C instead of a G at position 451;

(k) SEQ ID NO:1 having a C instead of an A at position 1093;

(l) SEQ ID NO:1 having a C instead of a G at position 1291;

(m) SEQ ID NO:1 having A at position 1493 deleted;

(n) SEQ ID NO:1 having a T instead of a C at position 2117;

(o) SEQ ID NO:1 having a C instead of an A at position 2411;

(p) SEQ ID NO:1 having an A instead of a G at position 4813;

(q) SEQ ID NO:1 having a G instead of a T at position 5868;

(r) SEQ ID NO:1 having a T instead of a C at position 5972;

(s) SEQ ID NO:1 having a T instead of a C at position 6328;

(t) SEQ ID NO:1 having a T instead of a G at position 7049;

(u) SEQ ID NO:1 having a C instead of a G at position 7491;

(v) SEQ ID NO:1 having a G instead of an A at position 9537;

(w) SEQ ID NO:1 having a T instead of an A at position 10204;

(x) SEQ ID NO:1 having a G instead of a C at position 10298;

(y) SEQ ID NO:1 having a G instead of an A at position 10462;

(z) SEQ ID NO:1 having an A instead of a G at position 203;

(aa) SEQ ID NO:1 having an A instead of a C at position 1342;

(bb) SEQ ID NO:1 having a C instead of a T at position 2457;

(cc) SEQ ID NO:1 having a G instead of an A at position 3199;

(dd) SEQ ID NO:1 having a G instead of an A at position 3624;

(ee) SEQ ID NO:1 having a G instead of an A at position 3668;

(ff) SEQ ID NO:1 having a C instead of a T at position 4035;

(gg) SEQ ID NO:1 having a G instead of an A at position 7470;

(hh) SEQ ID NO:1 having a G instead of an A at position 1593;

(ii) SEQ ID NO:1 having an A instead of a G at position 4296;

(jj) SEQ ID NO:1 having a G instead of an A at position 5691;

(kk) SEQ ID NO:1 having a G instead of an A at position 6051;

(ll) SEQ ID NO:1 having a C instead of a T at position 6828; and

(mm) SEQ ID NO:1 having a C instead of a T at position 6921.

11. A replicative cloning vector which comprises the isolated DNA molecule of claim 1, or at least 15 contiguous

12. A replicative cloning vector which comprises the isolated DNA molecule of claim 2, or at least 15 contiguous

13. A replicative cloning vector which comprises the isolated DNA molecule of claim 3, or at least 15 contiguous

14. A replicative cloning vector which comprises the isolated DNA molecule of claim 6, or at least 15 contiguous

15. A replicative cloning vector which comprises the isolated DNA molecule of claim 7, or at least 15 contiguous

16. An expression vector which comprises the isolated DNA of claim 1, or at least 15 contiguous nucleotides of

17. An expression vector which comprises the isolated DNA of claim 2, or at least 15 contiguous nucleotides of

18. An expression vector which comprises the isolated DNA of claim 3, or at least 15 contiguous nucleotides of

19. An expression vector which comprises the isolated DNA of claim 6, or at least 15 contiguous nucleotides of

20. An expression vector which comprises the isolated DNA of claim 7, or at least 15 contiguous nucleotides of

21. An isolated host cell transformed with the expression vector of claim 16.

22. An isolated host cell transformed with the expression vector of claim 17.

23. An isolated host cell transformed with the expression vector of claim 18.

24. An isolated host cell transformed with the expression vector of claim 19.

25. An isolated host cell transformed with the expression vector of claim 20.

26. A method of producing recombinant BRCA2 polypeptide which comprises culturing the cells of claim 21 und

27. A method of producing recombinant BRCA2 polypeptide which comprises culturing the cells of claim 22 und

28. A method of producing recombinant BRCA2 polypeptide which comprises culturing the cells of claim 23 und

29. A pair of single-stranded DNA primers of at least 15 nucleotides in length for determination of the nucleotide sequence or at least 15 contiguous nucleotides of the BRCA2 gene.

30. The pair of primers of claim 29 wherein said BRCA2 gene has the nucleotide sequence set forth in SEQ ID I

DESCRIPTION

CROSS REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of application Ser. No. 08/585,391, filed on 11 Jan. 1996, now abandoned, which i of application Ser. No. 08/573,779, filed on 18 Dec. 1995, now abandoned, all of which are incorporated herein by referenc

FIELD OF THE INVENTION

The present invention relates generally to the field of human genetics. Specifically, the present invention relates to method relates to germline mutations in the BRCA2 gene and their use in the diagnosis of predisposition to breast cancer. The pres gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to invention relates to the screening of the BRCA2 gene for mutations, which are useful for diagnosing the predisposition to b

The publications and other materials used herein to illuminate the background of the invention, and in particular, cases to p

BACKGROUND OF THE INVENTION

The genetics of cancer is complicated, involving multiple dominant, positive regulators of the transformed state (oncogenes
increase beyond fifty (Knudson, 1993).

The involvement of so many genes underscores the complexity of the growth control mechanisms that operate in cells to m
mutations are in the H-ras gene, found in 10-15% of all solid tumors (Anderson et al., 1992). The most frequently mutated t
to all transformed cells, the dream of a "magic bullet" that can destroy or revert cancer cells while leaving normal tissue unh

The tumor suppressor genes which have been cloned and characterized influence susceptibility to: 1) Retinoblastoma (RB
type 2A (MEN2A); and 9) Melanoma (CDKN2).

Tumor suppressor loci that have been mapped genetically but not yet isolated include genes for: Multiple endocrine neopla
sclerosis 2 (TSC2). The tumor suppressor genes that have been characterized to date encode products with similarities to
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 mutate

One of the hallmarks of several tumor suppressor genes characterized to date is that they are deleted at high frequency in
either because of a preexisting inherited mutation, or because of a secondary sporadic mutation.

Breast cancer is one of the most significant diseases that affects women. At the current rate, American women have a 1 in
Ovarian cancer, although less frequent than breast cancer, is often rapidly fatal and is the fourth most common cause of ca
Breast cancer has been subdivided into two types, early-age onset and late-age onset, based on an inflection in the age-sp

The BRCA1 gene has been isolated (Futreal et al., 1994; Miki et al., 1994) following an intense effort following its mapping
but confers a lower risk of ovarian cancer. The remaining susceptibility to early-onset breast cancer is divided between as-
risk for breast cancer (Swift et al., 1976; Swift et al., 1991). Late-age onset breast cancer is also often familial although the

Breast cancer has long been recognized to be, in part, a familial disease (Anderson, 1972). Numerous investigators have e
Bishop et al., 1988; Newman et al., 1988; Claus et al., 1991). Recent results demonstrate that at least three loci exist which
responsible for the unmapped residual. Hall et al. (1990) indicated that the inherited breast cancer susceptibility in kindreds

Most strategies for cloning the chromosome 13-linked breast cancer predisposing gene (BRCA2) require precise genetic lo
cancerous. However, cells that contain one wild type BRCA2 allele and one predisposing allele may occasionally suffer los
model, predisposing alleles of BRCA2 are recessive, yet susceptibility to cancer is inherited in a dominant fashion: women
loci known as tumor suppressors or antioncogenes, a class of genes that includes the retinoblastoma gene and neurofibro

A second possibility is that BRCA2 predisposing alleles are truly dominant; that is, a wild type allele of BRCA2 cannot over
predisposed individuals would undergo some other stochastic change(s) leading to cancer.

If BRCA2 predisposing alleles are recessive, the BRCA2 gene is expected to be expressed in normal mammary tissue but
be expressed in breast tumor cells.

The chromosome 13 linkage of BRCA2 was independently confirmed by studying fifteen families that had multiple cases of
D13S289 and D13S267, placing BRCA2 in a physical region defined by 13q12--13. The size of these regions and the unce
females. However, BRCA2 does not appear to confer a substantially elevated risk of ovarian cancer, although it does appe

Identification of a breast cancer susceptibility locus would permit the early detection of susceptible individuals and greatly i
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 method
invention relates to germline mutations in the BRCA2 gene and their use in the diagnosis of predisposition to breast cancer
gene in other human cancers and their use in the diagnosis and prognosis of human cancers. The invention also relates to
invention relates to the screening of the BRCA2 gene for mutations, which are useful for diagnosing the predisposition to b

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows a schematic map of STSs, P1s, BACs and YACs in the BRCA2 region.

FIG. 2 shows the sequence-space relationship between the cDNA clones, hybrid selected clones, cDNA PCR products and
screening a pool of human testis and HepG2 cDNA libraries with hybrid selected clone GT 713. The sequence 1-BR:CG02
selected clones, genomic sequence in the public domain and radioactive DNA sequencing gels. hlybrid selected clones loc
gland, placenta, testis and HepG2 cDNA libraries with the exon trapped clones wXBF1B8, wXPF1A5 and wXBF1B6. The c

FIGS. 3A-3D show the DNA sequence of the BRCA2 gene (which is also set forth in SEQ ID NO: 1).

FIG. 4 shows the genomic organization of the BRCA2 gene. The exons (boxes and/or vertical lines) are parsed across the
and 92M18.01289 actually overlap. Distances between the other genomic sequences are not known. Neither the public da

FIGS. 5A-5D show a loss of heterozygosity (LOH) analysis of primary breast tumors. Alleles of STR markers are indicated
on the abscissa. N is for normal (FIGS. 5A and 5C) and T is for tumor (FIGS. 5B and 5D).

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates generally to the field of human genetics. Specifically, the present invention relates to method
invention relates to germline mutations in the BRCA2 gene and their use in the diagnosis of predisposition to breast cancer
gene in other human cancers and their use in the diagnosis and prognosis of human cancers. IThe invention also relates to
invention relates to the screening of the BRCA2 gene for mutations, which are useful for diagnosing the predisposition to b

The present invention provides an isolated polynucleotide comprising all, or a portion of the BRCA2 locus or of a mutated B
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 BRCA2 locus
said portion of the BRCA2 locus. The method is useful for either diagnosis of the predisposition to cancer or the diagnosis

The present invention also provides isolated antibodies, preferably monoclonal antibodies, which specifically bind to an iso

The present invention also provides kits for detecting in an analyte a polynucleotide comprising a portion of the BRCA2 loc

The present invention further provides methods of preparing a polynucleotide comprising polymerizing nucleotides to yield
the BRCA2 locus.

The present invention further provides methods of screening the BRCA2 gene to identify mutations. Such methods may fur
identifying mutations for use in either diagnosis of the predisposition to cancer or the diagnosis or prognosis of cancer.

The present invention further provides methods of screening suspected BRCA2 mutant alleles to identify mutations in the B

In addition, the present invention provides methods of screening drugs for cancer therapy to identify suitable drugs for rest

Finally, the present invention provides the means necessary for production of gene-based therapies directed at cancer cell
reconstituted. Therapeutic agents may also take the form of polypeptides based on either a portion of, or the entire protein

It is a discovery of the present invention that the BRCA2 locus which predisposes individuals to breast cancer, is a gene en
it is a discovery of the present invention that somatic mutations in the BRCA2 locus are also associated with breast cancer
the non-coding sequence.

Starting from a region on human chromosome 13 of the human genome, which has a size estimated at about 6 million bas

The region containing the BRCA2 locus was identified using a variety of genetic techniques. Genetic mapping techniques i
the BRCA2 gene. A region which contains the BRCA2 locus is physically bounded by the markers D13S289 and D13S267

The use of the genetic markers provided by this invention allowed the identification of clones which cover the region from a
construction of a contig from a subset of the clones. These P1s, YACs and BACs provide the basis for cloning the BRCA2
The isolation was done using software trapping (a computational method for identifying sequences likely to contain coding
used to obtain sequences of loci expressed in breast and other tissue. These candidate loci were analyzed to identify sequ
The present invention not only facilitates the early detection of certain cancers, so vital to patient survival, but also permits

Population Resources

Large, well-documented Utah kindreds are especially important in providing good resources for human genetic studies. Ea
kindreds large enough to confirm the presence of a susceptibility allele. Large sibships are especially important for studying
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 th

Utah's age-adjusted breast cancer incidence is 20% lower than the average U.S. rate. The lower incidence in Utah is prob

Genetic Mapping

Given a set of informative families, genetic markers are essential for linking a disease to a region of a chromosome. Such
polymorphisms based on short tandem repeats (STRs), especially repeats of CpA (Weber and May, 1989; Litt et al., 1989)

Genetic markers useful in searching for a genetic locus associated with a disease can be selected on an ad hoc basis, by
to determine the ideal distance between genetic markers of a given degree of polymorphism, then selecting markers from
which are detected by amplification of the target nucleic acid sequence using PCR; such markers are highly informative, e

Once linkage has been established, one needs to find markers that flank the disease locus, i.e., one or more markers prox
as shown in the Examples.

Genetic mapping is usually an iterative process. In the present invention, it began by defining flanking genetic markers aro
localize the BRCA2 locus as either distal or proximal to a specific genetic marker (Wooster et al., 1994).

The region surrounding BRCA2, until the disclosure of the present invention, was not well mapped and there were few ma
polymorphic and which mapped to the BRCA2 region.

Physical Mapping

Three distinct methods were employed to physically map the region. The first was the use of yeast artificial chromosomes

Yeast Artificial Chromosomes (YACs). Once a sufficiently small region containing the BRCA2 locus was identified, physica
distributed and contain approximately 50,000 YACs each. The YACs isolated were from these publicly accessible libraries
we selected since they would not have known which YACs were within, and which YACs outside of, the smallest region co

P1 and BAC Clones. In the present invention, it is advantageous to proceed by obtaining P1 and BAC clones to cover this
with which the DNA of interest can be manipulated, and improves the signal-to-noise ratio of hybridization assays.

P1 and BAC clones are obtained by screening libraries constructed from the total human genome with specific sequence t

These P1 and BAC clones can be compared by interspersed repetitive sequence (IRS) PCR and/or restriction enzyme dig

overlapping contiguous set of clones which covers the region but is not excessively redundant, referred to herein as a "min

P1 clones (Stemberg, 1990; Sternberg et al., 1990; Pierce et al., 1992; Shizuya et al., 1992) were isolated by Genome Scie
permitted the covering of the genomic region with an independent set of clones not derived from YACs. This guards agains

Gene Isolation.

There are many techniques for testing genomic clones for the presence of sequences likely to be candidates for the coding

(a) Zoo blots. The first technique is to hybridize cosmids to Southern blots to identify DNA sequences which are evolutiona
variety of species are commercially available (Clonetech, Cat. 7753-1).

(b) Identifying HTF islands. The second technique involves finding regions rich in the nucleotides C and G, which often occ

(c) Exon trapping. The third technique is exon trapping, a method that identifies sequences in genomic DNA which contain
selection of RNA sequences which are flanked by functional 5' and/or 3' splice sites. The products of the exon amplification
trapping.

(d) Hybridizing cDNA to P1s, BACs or YACs. The fourth technique is a modification of the selective enrichment technique w
modified for the present purpose, involves binding DNA from the region of BRCA2 present in a YAC to a column matrix and
DNA.

(e) Identification of cDNAs. The fifth technique is to identify cDNAs that correspond to the BRCA2 locus. Hybridization prob

Another variation on the theme of direct selection of cDNA can be used to find candidate genes for BRCA2 (Lovett et al., 1
binding sites for primers in subsequent PCR amplification reactions using biotinylated primers. Target cDNA is generated f
adapters. These adapters serve as amplification sites for PCR. The target and probe sequences are denatured and mixed
high stringency and the retained cDNAs are eluted and amplified by PCR. The selected cDNA is subjected to further round

Testing the cDNA for Candidacy

Proof that the cDNA is the BRCA2 locus is obtained by finding sequences in DNA extracted from affected kindred member
non-kindred individuals with breast cancer then in individuals in the general population. Finally, since tumors often mutate s
tissue. Whether one is comparing BRCA2 sequences from tumor tissue to BRCA2 alleles from the germline of the same in
These mutations can take a number of forms. The most severe forms would be frame shift mutations or large deletions wh
which would have a significant effect on the protein produced, such as changes to or from a cysteine residue, from a basic
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 BRCA2 locus is dete
including deletions, insertions and point mutations in the coding and noncoding regions. Deletions may be of the entire gen

inherited in the germline. Germline mutations can be found in any of a body's tissues and are inherited. If only a single alle
information. A BRCA2 allele which is not deleted (e.g., found on the sister chromosome to a chromosome carrying a BRCA
product. However, mutations leading to non-functional gene products would also lead to a cancerous state. Point mutationa
BRCA2 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,

Predisposition to cancers, such as breast cancer, and the other cancers identified herein, can be ascertained by testing an
body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can b
discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual seq
rarely missed. Another approach is the single-stranded conformation polymorphism assay (SSCA) (Orita et al., 1989). This
increased throughput possible with SSCA makes it an attractive, viable alternative to direct sequencing for mutation detect
two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heterodu
which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such
recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific ol

In order to detect the alteration of the wild-type BRCA2 gene in a tissue, it is helpful to isolate the tissue free from surround
cytometry. These techniques, as well as other techniques for separating tumor cells from normal cells, are well known in th

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southe
hybridizing fragments (differing in length from control DNA when probed with sequences near or including the BRCA2 locu

Detection of point mutations may be accomplished by molecular cloning of the BRCA2 allele(s) and sequencing the allele(s
then be determined.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility
al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotid
BRCA2 mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS
restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score a

Such a method is particularly useful for screening relatives of an affected individual for the presence of the BRCA2 mutatio

In the first three methods (SSCA, DGGE and RNase protection assay), a new electrophoretic band appears. SSCA detects
DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing g
protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type seque

Mismatches according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100%
sensitive than sequencing, they are simpler to perform on a large number of tumor samples. An example of a mismatch cle

and either mRNA or DNA isolated from the tumor tissue are annealed (hybridized) together and subsequently digested wit
electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which i
BRCA2 mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mism

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cot
riboprobes or DNA probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see be

DNA sequences of the BRCA2 gene which have been amplified by use of PCR may also be screened using allele-specific
of the BRCA2 gene sequence. By use of a battery of such allele-specific probes, PCR amplification products can be scree
probe under stringent hybridization conditions indicates the presence of the same mutation in the tumor tissue as in the all

The most definitive test for mutations in a candidate locus is to directly compare genomic BRCA2sequences from cancer p

Mutations from cancer patients falling outside the coding region of BRCA2 can be detected by examining the non-coding r
abnormal size or abundance in cancer patients as compared to control individuals.

Alteration of BRCA2 mRNA expression can be detected by any techniques known in the art. These include Northern blot a
BRCA2 protein. For example, monoclonal antibodies immunoreactive with BRCA2 can be used to screen a tissue. Lack of
in the art. TIhese include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an alte
a mutant BRCA2 gene product indicates alteration of a wild-type BRCA2 gene.

Mutant BRCA2 genes or gene products can also be detected in other human body samples, such as serum, stool, urine ar
addition, the BRCA2 gene product itself may be secreted into the extracellular space and found in these body samples eve
such body samples for mutant BRCA2 genes or gene products.

The methods of diagnosis of the present invention are applicable to any tumor in which BRCA2 has a role in tumorigenesis

The primer pairs of the present invention are useful for determination of the nucleotide sequence of a particular BRCA2 all
these primers allows synthesis of all of the nucleotides of the BRCA2 gene coding sequences, i.e., the exons. The set of p
mutant allele as a template.

In order to facilitate subsequent cloning of amplified sequences, primers may have restriction enzyme site sequences appe
well known in the art. The primers themselves can be synthesized using techniques which are well known in the art. Gene
addition to those disclosed below, 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 South
with the BRCA2 gene or mRNA using other techniques.

It has been discovered that individuals with the wild-type BRCA2 gene do not have cancer which results from the BRCA2 a
or altered function, directly correlates to an increased risk of cancer. In order to detect a BRCA2 gene mutation, a biologica

described above. The mutant alleles are then sequenced to identify the specific mutation of the particular mutant allele. Alt
mutations, especially those which lead to an altered function of the BRCA2 protein, are then used for the diagnostic and pr

Definitions

The present invention employs the following definitions:

"Amplification of Polynucleotides" utilizes methods such as the polymerase chain reaction (PCR), ligation amplification (or l
PCR); and Wu et al., 1989a (for LCR). Reagents and hardware for conducting PCR are commercially available. Primers us
amplification may be sequenced directly. Alternatively, but less desirably, the amplified sequence(s) may be cloned prior to

"Analyte polynucleotide" and "analyte strand" refer to a single- or double-stranded polynucleotide which is suspected of con

"Antibodies." The present invention also provides polyclonal and/or monoclonal antibodies and fragments thereof, and imm
thereof. The term "antibody" is used both to refer to a homogeneous molecular entity, or a mixture such as a serum produc
rabbits. Rabbit sera is tested for immunoreactivity to the BRCA2 polypeptide or fragment. Monoclonal antibodies may be m
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 t
appropriate target immune system, typically mouse or rabbit, is selected. Substantially purified antigen is presented to the i
course, other species may be substituted for mouse or rabbit. Polyclonal antibodies are then purified using techniques kno

An immunological response is usually assayed with an immunoassay. Normally, such immunoassays involve some purifica

Monoclonal antibodies with affinities of 10^{-8} M^{-1} or preferably 10^{-9} to 10^{-1} M^{-1} or stronger will typically be made by standard p
excised and individual spleen cells fused, typically, to immortalized myeloma cells under appropriate selection conditions. T

Other suitable techniques involve in vitro exposure of lymphocytes to the antigenic polypeptides, or alternatively, to selectio
joining, either covalently or non-covalently, a substance which provides for a detectable signal. A wide variety of labels and
magnetic particles and the like. Patents teaching the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939

"Binding partner" refers to a molecule capable of binding a ligand molecule with high specificity, as for example, an antigen
hybridization) under the isolation conditions. Specific binding partners are known in the art and include, for example, biotin
about 15 bases in length, and may be at least 40 bases in length. The polynucleotides may be composed of DNA, RNA, or

A "biological sample" refers to a sample of tissue or fluid suspected of containing an analyte polynucleotide or polypeptide
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 cla

"Encode". A polynucleotide is said to "encode" a polypeptide if, in its native state or when manipulated by methods well kno
can be deduced therefrom.

"Isolated" or "substantially pure". An "isolated" or "substantially pure" nucleic acid (e.g., an RNA, DNA or a mixed polymer)
embraces a nucleic acid sequence or protein which has been removed from its naturally occurring environment, and incluc

"BRCA2 Allele" refers to normal alleles of the BRCA2 locus as well as alleles carrying variations that predispose individuals

"BRCA2 Locus," "BRCA2 Gene," "BRCA2 Nucleic Acids" or "BRCA2 Polynucleotide" each refer to polynucleotides, all of w
initiation and/or progression of other types of tumors. The locus is indicated in part by mutations that predispose individuals
The BRCA2 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 BRCA2 polypeptide, fragment, homol
substantial homology with a natural BRCA2-encoding gene or a portion thereof. The coding sequence for a BRCA2 polype

The polynucleotide compositions of this invention include RNA, cDNA, genomic DNA, synthetic forms, and mixed polymers
example, labels, methylation, substitution of one or more of the naturally occurring nucleotides with an analog, internucleot
polypeptides), intercalators (e.g., acridine, psoralen, etc.), chelators, alkylators, and modified linkages (e.g., alpha anomeri
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 BRCA2 region. The recombinant co
polynucleotide of genomic, cDNA, semi-synthetic, or synthetic origin which, by virtue of its origin or manipulation, 1) is not a

Therefore, recombinant nucleic acids comprising sequences otherwise not naturally occurring are provided by this inventio

cDNA or genomic libraries of various types may be screened as natural sources of the nucleic acids of the present inventio
for the desired proteins. Phage libraries are normally preferred, but other types of libraries may be used. Clones of a library

The DNA sequences used in this invention will usually comprise at least about five codons (nucleotides), more usually at le
specifically with a BRCA2-encoding sequence.

Techniques for nucleic acid manipulation are described generally, for example, in Sambrook et al., 1989 or Ausubel et al.,
Promega Biotec, U. S. Biochemicals, New England Nuclear, and a number of other sources. The recombinant nucleic acid
probes. See, GenBank, National Institutes of Health.

"BRCA2 Region" refers to a portion of human chromosome 13 bounded by the markers tdj3820 and YS-G-B10T. This regi

As used herein, the terms "BRCA2 locus," "BRCA2 allele" and "BRCA2 region" all refer to the double-stranded DNA compr

As used herein, a "portion" of the BRCA2 locus or region or allele is defined as having a minimal size of at least about eigh

"BRCA2 protein" or "BRCA2 polypeptide" refer to a protein or polypeptide encoded by the BRCA2 locus, variants or fragme
This term also does not refer to, or exclude modifications of the polypeptide, for example, glycosylations, acetylations, phos
as other modifications known in the art, both naturally and non-naturally occurring. Ordinarily, such polypeptides will be at l
stringency conditions, to BRCA2-encoding nucleic acids and closely related polypeptides or proteins retrieved by antisera t

The length of polypeptide sequences compared for homology will generally be at least about 16 amino acids, usually at lea

"Operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to fur

"Probes" . Polynucleotide polymorphisms associated with BRCA2 alleles which predispose to certain cancers or are assoc
that the probes will be perfectly complementary to the target sequence, stringent conditions will be used. Hybridization strin
that is, which minimize noise. Since such indications identify neutral DNA polymorphisms as well as mutations, these indica

Probes for BRCA2 alleles may be derived from the sequences of the BRCA2 region or its cDNAs. The probes may be of an
the range of about 8-30 base pairs, since the hybrid will be relatively stable under even stringent conditions. If some degre

The probes will include an isolated polynucleotide attached to a label or reporter molecule and may be used to isolate othe
using homologous is polynucleotides. Alternatively, polynucleotides encoding these or similar polypeptides may be synthes
Mutations may be introduced to modify the properties of the polypeptide, perhaps to change ligand-binding affinities, interc

Probes comprising synthetic oligonucleotides or other polynucleotides of the present invention may be derived from natura

Portions of the polynucleotide sequence having at least about eight nucleotides, usually at least about 15 nucleotides, and
tissue.

"Protein modifications or fragments" are provided by the present invention for BRCA2 polypeptides or fragments thereof wh
acetylation, carboxylation, phosphorylation, glycosylation, ubiquitination, labeling, e.g., with radionuclides, and various enzy

A variety of methods for labeling polypeptides and of substituents or labels useful for such purposes are well known in the a
labeled ligand. The choice of label depends on the sensitivity required, ease of conjugation with the primer, stability require

Besides substantially full-length polypeptides, the present invention provides for biologically active fragments of the polype
system, as well as sharing of immunological epitopes for binding, serving as either a competitor or substitute antigen for an
consists of at least five such amino acids, and more usually consists of at least 8-10 such amino acids. Methods of determi

For immunological purposes, tandem-repeat polypeptide segments may be used as immunogens, thereby producing highl

The present invention also provides for fusion polypeptides, comprising BRCA2 polypeptides and fragments. Homologous
properties or activities of the derivative proteins. For example, ligand-binding or other domains may be "swapped" between
galactosidase, trpE, protein A, β-lactamasc, alpha amylase, alcohol dehydrogenase and yeast alpha mating factor. See, e.

Fusion proteins will typically be made by either recombinant nucleic acid methods, as described below, or may be chemica

"Protein purification" refers to various methods for the isolation of the BRCA2 polypeptides from other biological material, s
by the present invention. Various methods of protein purification are well known in the art, and include those described in D

The terms "isolated", "substantially pure", and "substantially homogeneous" are used interchangeably to describe a protein
substantially pure protein will typically comprise about 60 to 90% w/w of a protein sample, more usually about 95%, and pr
polypeptide band upon staining the gel. For certain purposes, higher resolution may be provided by using HPLC or other m

A BRCA2 protein is substantially free of naturally associated components when it is separated from the native contaminant
associated components. A protein may also be rendered substantially free of naturally associated components by isolation

A polypeptide produced as an expression product of an isolated and manipulated genetic sequence is an "isolated polypep

"Recombinant nucleic acid" is a nucleic acid which is not naturally occurring, or which is made by the artificial combination
techniques. Such is usually done to replace a codon with a redundant codon encoding the same or a conservative amino a

"Regulatory sequences" refers to those sequences normally within 100 kb of the coding region of a locus, but they may als

"Substantial homology or similarity". A nucleic acid or fragment thereof is "substantially homologous" ("or substantially simi
usually at least about 70%, more usually at least about 80%, preferably at least about 90%, and more preferably at least ab

Alternatively, substantial homology or (similarity) exists when a nucleic acid or fragment thereof will hybridize to another nu
specificity occurs. Typically, selective hybridization will occur when there is at least about 55% homology over a stretch of a
stretches, and in certain embodiments will often be over a stretch of at least about nine nucleotides, usually at least about 2

Nucleic acid hybridization will be affected by such conditions as salt concentration, temperature, or organic solvents, in add
conditions will generally include temperatures in excess of 30° C., typically in excess of 37° C., and preferably in excess of
paramneter. See, e.g., Wetmur & Davidson, 1968.

Probe sequences may also hybridize specifically to duplex DNA under certain conditions to form triplex or other higher ord

The terms "substantial homology" or "substantial identity", when referring to polypeptides, indicate that the polypeptide or p

"Substantially similar function" refers to the function of a modified nucleic acid or a modified protein, with reference to the w
have an altered amino acid sequence and/or may contain modified amino acids. In addition to the similarity of function, the
Alternatively, the similarity of function (activity) of the modified polypeptide may be higher than the activity of the wild-type B
techniques. A nucleic acid with a function substantially similar to the wild-type BRCA2 gene function produces the modified

Homology, for polypeptides, is typically measured using sequence analysis software. See, e.g., the Sequence Analysis So

to various substitutions, deletions and other modifications. Conservative substitutions typically include substitutions within t

A polypeptide "fragment," "portion" or "segment" is a stretch of amino acid residues of at least about five to seven contiguo

The polypeptides of the present invention, if soluble, may be coupled to a solid-phase support, e.g., nitrocellulose, nylon, c

"Target region" refers to a region of the nucleic acid which is amplified and/or detected. The term "target sequence" refers t

The practice of the present invention employs, unless otherwise indicated, conventional techniques of chemistry, molecula
and materials for human gene mapping, including mapping of human chromosome 13, is provided, e.g., in White and Lalou

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

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natur
Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may
described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite m
obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and ann

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system
encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating seque
mRNA stabilizing sequences. Secretion signals may also be included where appropriate, whether from a native BRCA2 pr
may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Samb

An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may
et al., 1988. Many useful vectors are known in the art and may be obtained from such vendors as Stratagene, New Englan
metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate d
promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molo
made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expre

While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of t

Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or
substances, e.g. ampicillin, neomycin, methotrexate, etc.; b) complement auxotrophic deficiencies, or c) supply critical nutr
art.

The vectors containing the nucleic acids of interest can be transcribed in vitro, and the resulting RNA introduced into the ho
electroporation; transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other su
the polynucleotides into the host cell by any method known in the art, including, inter alia, those described above, will be re

Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the BRCA2
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 sp
are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be app

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same
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 p

Antisense polynucleotide sequences arc useful in preventing or diminishing the expression of the BRCA2 locus, as will be
control of a promoter in an antisense orientation and introduced into a cell. Expression of such an antisense construct withi

The probes and primers based on the BRCA2 gene sequences disclosed herein are used to identify homologous BRCA2 g
isolated.

Methods of Use: Nucleic Acid Diagnosis and Diagnostic Kits

In order to detect the presence of a BRCA2 allele predisposing an individual to cancer, a biological sample such as blood i
sample of the lesion is prepared and analyzed for the presence or absence of mutant alleles of BRCA2. Results of these te
to health care providers or to private individuals for self-diagnosis.

Initially, the screening method involves amplification of the relevant BRCA2sequences. In another preferred embodiment o
can detect target sequences with a high level of sensitivity.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polym
increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic a

When the probes are used to detect the presence of the target sequences (for example, in screening for cancer susceptibil
denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually m
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 sequen
stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only
temperature, ionic strength, base composition, probe length, and concentration of formamide. These factors are outlined in
sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may
labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescen

hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these

As noted above, non-PCR based screening assays are also contemplated in this invention. An exemplary non-PCR based
enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridizat
development or luminescent output resulting in a 10^3 -10^6 increase in sensitivity. For an example relating to preparation of

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (su
application. Allele-specific probes are also contemplated within the scope of this example, and exemplary allele specific pro

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conju
labeling nucleic acid probes according to this embodiment see Martin et al., 1990. In a second example, the small ligand is
probes and their use in biotin-avidin based assays see Rigby et al., 1977 and Nguyen et al., 1992.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a c
probes is alternatively 2, 3, or 5 different nucleic acid probe sequences. In another example, to detect the presence of muta
alterations in BRCA2. In this embodiment, any number of probes can be used, and will preferably include probes correspon
and those that have the BRCA2 regions shown in SEQ ID NO:1 and FIG. 3, both 5' and 3' to the mutation site.

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 BRCA2 polypeptide. Suc
antibodies may be prepared as discussed above under the heading "Antibodies" and as further shown in Examples 9 and 1
immunoprecipitate BRCA2 proteins from solution as well as react with BRCA2 protein on Western or immunoblots of polya

Preferred embodiments relating to methods for detecting BRCA2 or its mutations include enzyme linked immunosorbent as
by David et al. in U.S. Pat. Nos. 4,376,110 and 4,486,530, hereby incorporated by reference, and exemplified in Example 9

Methods of Use: Drug Screening

This invention is particularly useful for screening compounds by using the BRCA2 polypeptide or binding fragment thereof i

The BRCA2 polypeptide or fragment employed in such a test may either be free in solution, affixed to a solid support, or bo
assays. Such cells, either in viable or fixed form, can be used for standard binding assays. One may measure, for example
interfered with by the agent being tested.

Thus, the present invention provides methods of screening for drugs comprising contacting such an agent with a BRCA2 p
by methods well known in the art. In such competitive binding assays the BRCA2 polypeptide or fragment is typically labele
BRCA2: ligand binding, respectively.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to

substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with BRCA2 polypeptide ar
to the polypeptide can be used to capture antibodies to immobilize the BRCA2 polypeptide on the solid phase.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of
more antigenic determinants of the BRCA2 polypeptide.

A further technique for drug screening involves the use of host eukaryotic cell lines or cells (such as described above) whic
to determine if the compound is capable of regulating the growth of BRCA2 defective cells.

Methods of Use: Rational Drug Design

The goal of rational drug design is to produce structural analogs of biologically active polypeptides of interest or of small m
polypeptide in vivo. See, e.g., Hodgson, 1991. In one approach, one first determines the three-dimensional structure of a p
regarding the structure of a polypeptide may be gained by modeling based on the structure of homologous proteins. An exa
replaced by Ala, and its effect on the peptide's activity is determined. Each of the amino acid residues of the peptide is ana

It is also possible to isolate a target-specific antibody, selected by a functional assay, and then to solve its crystal structure
pharmacologically active antibody. As a mirror image of a mirror image, the binding site of the anti-ids would be expected t

Thus, one may design drugs which have, e.g., improved BRCA2 polypeptide activity or stability or which act as inhibitors, a
crystallography. In addition, the knowledge of the BRCA2 protein sequence provided herein will guide those employing cor

Methods of Use: Gene Therapy

According to the present invention, a method is also provided of supplying wild-type BRCA2 function to a cell which carries
extrachromosomal. In such a situation, the gene will be expressed by the cell from the extrachromosomal location. If a gen
the wild-type BRCA2 gene or a part thereof is introduced into the mutant cell in such a way that it recombines with the endo
extrachromosomal maintenance are known in the art, and any suitable vector may be used. Methods for introducing DNA i
be used as model systems to study cancer remission and drug treatments which promote such remission.

As generally discussed above, the BRCA2 gene or fragment, where applicable, may be employed in gene therapy method
absent or diminished compared to normal cells. It may also be useful to increase the level of expression of a given BRCA2

Gene therapy would be carried out according to generally accepted methods, for example, as described by Friedman, 199
copy of the BRCA2 gene linked to expression control elements and capable of replicating inside the tumor cells, is prepare
any tumor cells that may have metastasized to other sites). If the transfected gene is not permanently incorporated into the

Gene transfer systems known in the art may be useful in the practice of the gene therapy methods of the present invention
and Kapikian, 1992; Quantin et al., 1992; Rosenfeld et al., 1992; Wilkinson et al., 1992; Stratford-Perricaudet et al., 1990),
and retroviruses of avian (Brandyopadhyay and Temin, 1984; Petropoulos et al., 1992), murine (Miller, 1992; Miller et al., 1

been based on disabled murine retroviruses.

Nonviral gene transfer methods known in the art include chemical techniques such as calcium phosphate coprecipitation (C
via liposomes (Felgner et al., 1987; Wang and Huang, 1989; Kaneda et al., 1989; Stewart et al., 1992; Nabel et al., 1990; L
et al., 1991a; Curiel et al., 1991b). Viral-mediated gene transfer can be combined with direct in vivo gene transfer using lipo
producer cells would then provide a continuous source of vector particles. This technique has been approved for use in hu

In an approach which combines biological and physical gene transfer methods, plasmid DNA of any size is combined with
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 lipo

Gene transfer techniques which target DNA directly to breast and ovarian tissues, e.g., epithelial cells of the breast or ovar
of the presence of the corresponding ligand receptors on the cell surface of the target cell/tissue type. One appropriate rec
binding and internalization of the DNA-protein complex occurs. To overcome the problem of intracellular destruction of DN

The therapy involves two steps which can be performed singly or jointly. In the first step, prepubescent females who carTy
treated individuals have reduced risk of breast cancer to the extent that the effect of the susceptible allele has been counte
effects on the breast of a full term pregnancy.

Methods of Use: Peptide Therapy

Peptides which have BRCA2 activity can be supplied to cells which carry mutant or missing BRCA2 alleles. The sequence
BRCA2-producing mammalian cells. In addition, the techniques of synthetic chemistry can be employed to synthesize BRC
in a microorganism or in vitro.

Active BRCA2 molecules can be introduced into cells by microinjection or by use of liposomes, for example. Alternatively, s
reversal of the neoplastic state. Other molecules with BRCA2 activity (for example, peptides, drugs or organic compounds)

Methods of Uise: Transformed Hosts

Similarly, cells and animals which carry a mutant BRCA2 allele can be used as model systems to study and test for substa
carry the mutation in the BRCA2 allele, as described above. After a test substance is applied to the cells, the neoplastically
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 c
disrupted by insertion or deletion mutation or other genetic alterations using conventional techniques (Capecchi, 1989; Vala
tumors must be assessed. If the test substance prevents or suppresses the growth of tumors, then the test substance is a

The present invention is described by reference to the following Examples, which are offered by way of illustration and are

EXAMPLE 1 Ascertain and Study Kindreds Likely to Have a Chromosome 13-Linked Breast Cancer Susceptibility Locus

Extensive cancer prone kindreds were ascertained from a defined population providing a large set of extended kindreds wi
opportunity for informative recombinants to occur within the small region being investigated. This vastly improved the chan

Each kindred was extended through all available connecting relatives, and to all informative first degree relatives of each p
reported in the kindred which were not confirmed in the Utah Cancer Registry were researched. Medical records or death c
and relatives of deceased cases so that the genotype of the deceased cases could be inferred from the genotypes of their

Kindreds which had three or more cancer cases with inferable genotypes were selected for linkage studies to chromosome
two sisters or a mother and her daughter with breast cancer. Additionally, kindreds which have been studied since 1980 as
kindreds were investigated and expanded in our clinic in the manner described above.

For each sample collected in these kindreds, DNA was extracted from blood or paraffin- embedded tissue blocks using sta
To aid in this effort, STR markers on chromosome 13 were developed by screening a chromosome specific cosmid library
published reports, or as part of the Breast Cancer Linkage Consortium, or from other investigators. All genotyping films we

LOD scores for each kindred were calculated for two recombination fraction values, 0.001 and 0.1. (For calculation of LOD
specific risks for breast cancer in non-gene carriers. Allele frequencies for the markers used for the LOD score calculations

Kindred 107 is the largest chromosome 13-linked breast cancer family reported to date by any group. The evidence of linka

In order to improve the characterization of our recombinants and define closer flanking markers, a dense map of this relativ
shows the location of ten markers used in the genetic analysis. Table 1 gives the LOD scores for linkage for each of the 19

TABLE 1

D13SKindred FBR MBR OV LOD Probability (2) 3820 4247 260
267 107* 22 3 2 5.06
82043* 2 1 1 0.86 0.98 6 30 3 12 7 10 5 8 4 122018 3 1 0 n.d. 0.90 9 12 7 3 8 3 6 6 5 8 937
1 0 n.d. 0.90 3 29 7 10 5 8 5 5 5 88003 2 1 0 n.d. 0.90 4 12 6 10 6 3 4 5 4 82367 6 0 1 0.4
0.36 0.84 9 10 6 4 6 3 7 5 8 82327 11 0 0 1.92 0.99 3 12 2 9 5 10 5 5 3 41019 2 2 0
segregating in the family). FBR = female breast cancer under 60 years. MBR = male breast cancer OV = ovarian cancer (2
to BRCA1.

Table 1 also gives the posterior probability of a kindred having a BRCA2 mutation based on LOD scores and prior probabil
recombinant in Kindred 107 with marker tdj3820 at the left boundary, and a second recombinant in Kindred 2043 with mark
been driven by BRCA2 itself, this deletion is referred to as the Schutte/Kern deletion in FIG. 1 (Schutte et al., 1995). The S

EXAMPLE 2 Development of Genetic and Physical Resources in the Region of Interest

To increase the number of highly polymorphic loci in the BRCA2 region, we developed a number of STR markers in our lab

STSs in the desired region were used to identify YACs which contained them. These YACs were then used to identify subc
number of repeats and/or are of near- perfect fidelity to the pattern. Both of these characteristics are known to increase the
primers complementary to the end of the repeat. Based on this unique sequence, a primer was made to sequence back ac
confirm their physical localization. New markers which satisfied these criteria were then typed in a set of unrelated individu

Using the procedure described above, novel STRs were found from these YACs which were both polymorphic and localize

EXAMPLE 3 Identification of Candidate cDNA Clones for the BRCA2 Locus by Genomic Analysis of the Contig Region

1. General Methods

Complete screen of the plausible region. The first method to identify candidate cDNAs, although labor intensive, used know
identify candidate cDNA clones for future analysis. The clones were screened for putative coding sequences by either of tw

The P1 clones to be analyzed were digested with a restriction enzyme to release the human DNA from the vector DNA. Th
buffer (Maniatis et al., 1982). The eluted Not I digested DNA (˜15 kb to 25 kb) was then digested with EcoRI restriction enz
(Boehringer-Mannheim, Cat. #1004760). The labeled DNA was spermine precipitated (add 100 μl TE, 5 μl 0.1 M spermine
per the manufacturer's instructions (Gibco/BRL, Cat. #5279SA). The C_o t-1 blocked probe was incubated on the filters in th
XAR-5 film with an intensifying screen. Thus, the blots were hybridized with either the pool of Eco-RI fragments from the in

The human DNA from clones in the region was isolated as whole insert or as EcoRI fragments and labeled as described ab

Most of the cDNA libraries used to date in our studies (libraries from normal breast tissue, breast tissue from a woman in h
vector, and is grown in C600Hfl bacterial host cells. Normal breast tissue and malignant breast tissue samples were isolate
selection of the final products which were then cloned into the Lambda Zap II vector, and grown in XL1-blue strain of bacte
(Clonetech Cat. HL11810), human placenta (Clonetech Cat 1075b), and human skeletal muscle (Clonetech Cat. HL1124b)

The cDNA libraries were plated with their host cells on NZCYM plates, and filter lifts are made in duplicate from each plate
which correspond to genes located within the candidate cosmid clone. cDNAs identified by this method were picked, replat
blot analysis and sequencing. Clones were either purified as plasmid through in vivo excision of the plasmid from the Lamb

The Southern blot analysis was performed in duplicate, one using the original genomic insert DNA as a probe to verify that
are unique were sequenced and the DNA analyzed to determine if the sequences represent known or unique genes. All cD
tumor RNAs. They are also analyzed by PCR on clones in the BRCA2 region to verify their location. To map the extent of t
analysis.

We have screened the normal breast, 8 month pregnant breast and fetal brain cDNA libraries with Eco RI fragments from c

Analysis of hybrid-selected cDNA. cDNA fragments obtained from direct selection were checked by Southern blot hybridiza
independent clones that overlapped.

The direct selection of cDNA method (Lovett et al., 1991; Futreal, 1993) is utilized with P1 and BAC DNA as the probe. The
primers. Target cDNA is generated from mRNA derived from tissue samples, e.g., breast tissue, by synthesis of either ranc
sequences are denatured and mixed with human C_o t-1 DNA to block repetitive sequences. Solution hybridization is carriec
selected cDNA is subjected to further rounds of enrichment before cloning into a plasmid vector for analysis.

HTF island analysis. A method for identifying cosmids to use as probes on the cDNA libraries was HTF island analysis. HT
dinucleotides. Enzymes known to be useful in HTF-island analysis are AscI, NotI, BssHII, EagI, SacII, NaeI, NarI, SmaI, ar

Analysis of candidate clones. One or more of the candidate genes generated from above were sequenced and the informa
amino acid sequences. This was accomplished using Genetic Data Environment (GDE) version 2.2 software and the Basic
workstations. Sequences reconstructed from collections of cDNA clones identified with the cosmids and P1s have been ge

Mutation screening. To screen for mutations in the affected pedigrees, two different approaches were followed. First, genor
amplified fragment will be larger than predicted from the cDNA sequence or will not be present in the amplified mixture. By
the kindreds.

A second approach that is much more rapid if the intron/exon structure of the candidate gene is complex involves sequenc
significant extent in lymphocytes, such experiments usually produce amplified fragments that can be sequenced directly wi

The products of such sequencing reactions were analyzed by gel electrophoresis to determine positions in the sequence th

Any sequence within the BRCA2 region that is expressed in breast is considered to be a candidate gene for BRCA2. Comp

2. Specific Methods

Hybrid selection. Two distinct methods of hybrid selection were used in this work.

Method 1: cDNA preparation and selection. Randomly primed cDNA was prepared from poly $(A)^+$ RNA of mammary gland,
DNA as described previously. (Parimoo et al., 1991; Rommens et al., 1994). Groups of two to four overlapping P1 and/or B
agarose gels, and ligated into pBluescript (Stratagene) that had been digested with EcoRI and treated with calf alkaline pho

Characterization of Retrieved cDNAs. 200 to 300 individual colonies from each ligation (from each 250 kbases of genomic
subsequently lysed with standard procedures. Initial analysis of the cDNA clones involved a prescreen for ribosomal seque

Approximately 10-25% of the clones were eliminated as they hybridized strongly with radiolabeled cDNA obtained from tot
clones by hybridization to restriction digests of DNAs of the starting clones, of a hamster hybrid cell line (GM10898A) that c
approximately 85% mapped appropriately to the starting clones.

Method 2 (Lovett et al., 1991): cDNA Preparation. Poly(A) enriched RNA from human mammary gland, brain, lymphocyte a

5'-(NH_2)-GTAGTGCAAGGCTCGAGAACNNNNNNNNNNNN! (SEQ ID NO:3) and Superscript II reverse transcriptase (Gibc

5'-(NH_2)-TGAGTAGAATTCTAACGGCCGTCATTGTTC (SEQ ID NO:4) annealed to

5'-GAACAATGACGGCCGTTAGAATTCTACTCA-(NH_2) (SEQ ID NO:5)! to their 5' ends (5' relative to mRNA) using T4 DN

Selection. cDNAs from mammary gland, brain, lymphocyte and stomach tissues were first amplified using a nested version

(RP.A: 5'-TGAGTAGAATTCTAACGGCCGTCAT) (SEQ ID NO:6) and

XPCR 5'-(PO_4)-GTAGTGCAAGGCTCGAGAAC (SEQ ID NO:7)! and purified by fractionation on Sepharose CL-4B. Selecti
Probe, cDNA and Cot-1 DNA were hybridized in 2.4M TEA-CL, 10 mM $NaPO_4$, 1 mM EDTA. Hybridized cDNAs were captu

RP.B: 5'-(PO_4)-TGAGTAGAATTCTAACGGCCGTCATTG (SEQ ID NO:8)! and XPCR, and size-selected on Sepharose CL
dephosphorylated HincII cut pUC18. Ligation products were transformed into XL2-Blue ultracompetent cells (Stratagene).

Analysis. Approximately 192 colonies for each single-probe selection experiment were amplified by colony PCR using vect
clones were sequenced in both directions using vector primers on an ABI 377sequencer.

Exon Trapping. Exon amplification was performed using a minimally overlapping set of BACs, P1s and PACs in order to iso
PstI or BamHI+ BglII and ligated into PstI or BamHI sites of the pSPL3 splicing vector. The exon amplification technique wa
filters, and analyzed for the presence of vector and repeat sequences by hybridization. Each clone insert was PCR amplifie
hybridization to cDNA libraries.

5' RACE. The 5' end of BRCA2 was identified by a modified RACE protocol called biotin capture RACE. Poly(A) enriched R

5' (NH_2) -GTAGTGCAAGGCTCGAGAACNNNN (SEQ ID NO:3)! and Superscript II reverse transcriptase (Gibco BRL). The
5'-CCTTCACACGCGTATCGATTAGTCACNNNNNNNN-(NH_2) (SEQ ID NO:9) annealed to 5'-(PO_4)-GTGACTAATCGATACG
5'-(B)-TTGAAGAACAACAGGACTTTCACTA! (SEQ ID NO:11) and a nested version of UCA UCP.A: 5'-CACCTTCACACG
GTTCGTAATTGTTGTTTTTATGTTCAG! (SEQ ID NO: 13) and a further nested version of UCA UCP.B: 5'-CCTTTCACAC

cDNA Clones. Human cDNA libraries were screened with ^{32}P-labeled hybrid selected or exon trapped clones. Phage elute

Northern Blots. Multiple Tissue Northern (MTN) filters, which are loaded with 2 tig per lane of poly(A)+ RNA derived from a
phosphate dehydrogenase (GAPDH) were used to probe the filters. Prehybridizations were at 42° C. in 50% formamide, 5×
0.1×SSC/0.1% SDS at 50° C.

RT-PCR Analysis. Ten μg of total RNA extracted from five human breast cancer cell lines (ZR-75-1, T-47D, MDA-MB-231,

5'-TTTGGATCATTTTCACACTGTC! (SEQ ID NO:15)! and Superscript II reverse transcriptase (Gibco BRL). Thereafter, the

5'-GTGCTCATAGTCAGAAATGAAG! (SEQ ID NO:16)! and mH20-1D05#RA (this is the primer pair that was used to island

PCR Amplification and Mutation Screening. All 26 coding exons of BRCA2 and their associated splice sites were amplified
11-6, 11-7 and 23 through 27) were amplified by a simple one-step method. The PCR conditions for those exons were: sin
carried out with the primers in the first two columns of Table 2 for 19 cycles as described above. Nested reamplification for
agarose gels using Qiaex beads (Qiagen). The purified products were analyzed by cycle sequencing with α-P^{32} dATP with
polymorphisms was carried out visually and confirmed on the other strand.

TABLE 2

TGTTCCCATCCTCACAGTAAG*.sup.(17) GTACTGGGTTTTTAGCAAGCA*.sup.(18) 3 GGTTAAAACTAAGG
GGGGGTAAAAAAAGGGGAA*.sup.(24) 6 GAGATAAGTCAGGTATGATT*.sup.(25) AATTGCCTGTATGAGG
GGACCTAGGTTGATTGCA*.sup.(31) GTCAAGAAAGGTAAGGTAA*.sup.(32)10-1 CTATGAGAAAGGTTGT
CATGTATACAGATGATGCCTAAG*.sup.(38)11-1 AACTTAGTGAAAAATATTTAGTGA.sup.(39) ATACATCT
TAGCTCTTTTGGGACAATTC*.sup.(44) U11-3 ATGGAAAAGAATCAAGATGTAT*.sup.(45)
CTTGCTGCTGTCTACCTG.sup.(49) AGTGGTCTTAAGATAGTCAT*.sup.(50)
AACGGACTTGCTATTTACTGA*.sup.(55) AGTACCTTGCTCTTTTTCATC*.sup.(56)11-7 CAGCTAGCGGGA
TTATTCTCGTTGTTTTCCTTA*.sup.(61)11-9 CCATTAAATTGTCCATATCTA*.sup.(62)
TGAGACTTTGGTTCCTAATAC*.sup.(66) AGTAACGAACATTCAGACCAG*.sup.(67
ACTCTTTCAAACATTAGGTCA*.sup.(71) TTGGAGAGGCAGGTGGAT.sup.(72) CTAT
A14 GAATACAAAACAGTTACCAGA.sup.(77) CACCACCAAAGGGGGAAA*.sup.(78)
ATGTTTTTGTAGTGAAGATTCT.sup.(83) TAGTTCGAGAGACAGTTAAG*.sup.(84) CA
C18 TTTTATTCTCAGTTATTCAGTG.sup.(89) GAAATTGAGCATCCTTAGTAA*.sup.(90)
TGAAAACTCTTATGATATCTGT*.sup.(9 4)20 TGAATGTTATATATGTGACTTTT*.sup.(95)
GCCAGAGAGTCTAAAACAG*.sup.(99) CTTTGGGTGTTTTATGCTTG*.sup.(100
ATCACTTCTTCCATTGCATC*.sup.(104) CCGTGGCTGGTAAATCTG*.sup.(105)24 CTGGTAGCTCCAACTA
ACTTACAGGAGCCACATAAC*.sup.(111)27 CTACATTAATTATGATAGGCTNCG**.sup.(112) GTACTAATGT
without an "*" were replaced by the internal nested primer for both the second round of PCR and sequencing. For large exo
EXAMPLE 4 Identification of BRCA2

Assembly of the full-length BRCA2sequence. The full-length sequence of BRCA2 was assembled by combination of severa
mRNA including the predicted translational start site was identified by a modified 5' RACE protocol (Stone et al., 1995). The
11 was identified by analysis of roughly 900 kb of genomic sequence in the public domain (ftp://genome.wust1.edu/ pub/gs
of nearly 5 kb was identified that was spanned by long ORFs. This sequence was linked together by island hopping experi
SEQ ID NO:1 and FIG. 3.

Structure of the BRCA2 gene and BRCA2 polypeptide. Conceptual translation of the cDNA revealed an ORE that began at
membrane- spanning regions. Like BRCA1, the BRCA2 protein is highly charged. Roughly one quarter of the residues are

The BRCA2 gene structure was determined by comparison of cDNA and genomic sequences. BRCA2 is composed of 27 e
BRCA2 appears to be unique, with no close homologs in the human genome.

Expression studies of BRCA2. Hybridization of labeled cDNA to human multiple tissue Northern filters revealed an 11-12 k
PCR experiments using a BRCA2 cDNA amplicon were performed on five breast and three prostate cancer cell line RNAs.
amplified more efficiently from breast than from thymus.

Germline mutations in BRCA2. Individuals from eighteen putative BRCA2 kindreds were screened for BRCA2 germline mu
linked BRCA2 haplotype. Each of the 18 kindreds has a posterior probability of harboring a BRCA2 mutation of at least 69
were screened for mutations in multiple individuals from nine kindreds using either cDNA or genomic DNA (Table 3). Indivi
junctions.

TABLE 3____

Effect____UT-107[1]20 18 2 3 5.06
4706 del4 11 1493 termination codon at 1502UT-2367[1]6 5 1 0 2.09 0.99 I
1.09 1.00 8525 delC 18 2766 termination codon at 2776CU-1592[2]8 4 0 0
2204[2]3 1 0 4 0.51 0.98 999 del5 9 257 termination codon at 273MS-075
0.39 0.79 NDUT-2263[2]3 2 0 1 nd 0.9 NDUT-2171[2]5 4 2 0 nd nd ND________________________________
cDNA sample available. IR inferred regulatory mutation nd not determined Ov Ovarian Cancer ND none detected FBC I

Sequence alterations were identified in 9 of 18 kindreds. All except one involved nucleotide deletions that altered the readi

A subset of kindreds was tested for transcript loss. cDNA samples were available for a group of nine kindreds, but three of
polymorphic sites was interpreted as evidence for a mutation leading to reduction in mRNA levels. In only one of the six ca
some mutations in the BRCA2 coding sequence may destabilize the transcript in addition to disrupting the protein sequenc

Role of BRCA2 in Cancer. Most tumor suppressor genes identified to date give rise to protein products that are absent, nor
Srivastava et al., 1993). A similar dominant negative mechanism of action has been proposed for some adenomatous poly
BRCA2 coding sequence is consistent with production of either dominant negative proteins or nonfunctional proteins.

EXAMPLE 5 Analysis of the BRCA2 Gene

The structure and function of BRCA2 gene are determined according to the following methods.

Biological Studies. Mammalian expression vectors containing BRCA2 cDNA are constructed and transfected into appropria
Phenotypic reversion in cultures (e.g., cell morphology, doubling time, anchorage-independent growth) and in animals (e.g

Molecular Genetics Studies. In vitro mutagenesis is performed to construct deletion mutants and missense mutants (by sin

Mechanism Studies. The ability of BRCA2 protein to bind to known and unknown DNA sequences is examined. Its ability to

The nature and functions of the partners are characterized. These partners in turn are targets for drug discovery.

Structural Studies. Recombinant proteins are produced in E. coli, yeast, insect and/or mammalian cells and are used in cry

EXAMPLE 6 Two Step Assay to Detect the Presence of BRCA2 in a Sample

Patient sample is processed according to the method disclosed by Antonarakis et al. (1985), separated through a 1% agar
pTZ18U. The phagemids are transformed into E. coli MV1190 infected with M13KO7 helper phage (Bio-Rad, Richmond, C

Blots are prehybridized for 15-30 min at 65° C. in 7% sodium dodecyl sulfate (SDS) in 0.5M $NaPO_4$. The methods follow th
$NaPO_4$ at 65° C., followed by two 30 min washes in 1% SDS, 40 mM $NaPO_4$ at 65° C.

Next the blots are rinsed with phosphate buffered saline (pH 6.8) for 5 min at room temperature and incubated with 0.2% c
Denhardt's solution (see Sambrook, et al., 1989). The buffer is removed and replaced with 50-75 $\mu l/cm^2$ fresh hybridization
C. and post hybridization washes are incubated at 45° C. as two 10 min washes in 6M urea, 1× standard saline citrate (SS

Blots are incubated for 10 min at room temperature with shaking in the substrate buffer consisting of 0.1M diethanolamine,
Rad). After a 20 min incubation at room temperature with shaking, the excess AMPPD solution is removed. The blot is exp

EXAMPLE 7 Generation of Polyclonal Antibody against BRCA2

Segments of BRCA2 coding sequence are expressed as fusion protein in E. coli. The overexpressed protein is purified by
al., 1993).

Briefly, a stretch of BRCA2 coding sequence selected from the sequence shown in FIG. 3 is cloned as a fusion protein in p
identification of the protein as the BRCA2 fusion product is verified by protein sequencing at the N-terminus. Next, the purif
followed by 100 μg of immunogen in PBS. Antibody containing serum is collected two weeks thereafter.

This procedure is repeated to generate antibodies against the mutant forms of the BRCA2 gene. These antibodies, in conju

EXAMPLE 8 Generation of Monoclonal Antibodies Specific for BRCA2

Monoclonal antibodies are generated according to the following protocol. Mice are immunized with immunogen comprising

The immunogen is mixed with an adjuvant. Each mouse receives four injections of 10 to 100 μg of immunogen and after th
are selected for hybridoma production.

Spleens are removed from immune mice and a single cell suspension is prepared (see Harlow and Lane, 1988). Cell fusion
Harlow and Lane, 1988. Cells are plated at a density of 2×10^5 cells/well in 96 well tissue culture plates. Individual wells are
subcloned to establish and confirm monoclonality.

Clones with the desired specificities are expanded and grown as ascites in mice or in a hollow fiber system to produce suff

EXAMPLE 9 Sandwich Assay for BRCA2

Monoclonal antibody is attached to a solid surface such as a plate, tube, bead, or particle. Preferably, the antibody is attac
room temperature. Next the sample fluid is decanted, and the solid phase is washed with buffer to remove unbound materi
the solid phase with the second antibody is incubated for two hrs at room temperature. The second antibody is decanted a

The amount of bound label, which is proportional to the amount of BRCA2 peptide/protein present in the sample, is quantit

EXAMPLE 10 The 6174delT Mutation is Common in Ashkenazi Jewish Women Affected by Breast Cancer

The 6174delT mutation (see Table 3) has been found to be present in many cases of Ashkenazi Jewish women who have
probands affected with breast cancer on or before 41 years of age with or without a family history of breast cancer. Inclusio
proband was affected with breast cancer between the ages of 41 and 51 with two or more second degree relatives affected
counseling clinics, with an effort to offer study participation to all eligible patients. Family history was obtained by a self-rep

Mutation Detection

The BRCA2 6174delT mutation was detected by amplifying genomic DNA from each patient according to standard polyme

BC11-RP: GGGAAGCTTCATAAGTCAGTC (SEQ ID NO: 115) (forward primer) and

BC11-LP: TTTGTAATGAAGCATCTGATACC (SEQ ID NO: 116) (reverse primer).

The reactions were performed in a total volume of 10.0 µl containing 20 ng DNA with annealing at 55° C. This produces a
The gels were then dried and autoradiographed. All the cases exhibiting the 1 bp deletion were sequenced to confirm the 6
noncoding strand was sequenced. For one set the PCR primers were:

TD-SFB: AATGATGAATGTAGCACGC (SEQ ID NO: 117) (forward primer) and

CGORF-RH: GTCTGAATGTTCGTTACT (SEQ ID NO: 118) (reverse primer).

This results in an amplified product of 342 bp in wild-type and 341 bp for samples containing the 6174delT mutation. For th
in a fragment of 183 bp in wild-type samples and 182 bp in samples containing the 6174delT mutation. This was sequence

Results

Six out of eighty women of Ashkenazi Jewish ancestry with breast cancer before the age of 42 had the 6174delT mutation.
of the study. Four of the six cases with the 6174delT mutation had a family history of breast or ovarian cancer in a first or s

cancer at age 42-50 and a history of at least one additional relative affected with breast or ovarian cancer provided an addi
From the data presented, and assuming a penetrance similar to BRCA1 mutations (Offit et al., 1996; Langston et al., 1996)
precise estimate of the carrier frequency of the 6174delT mutation in individuals of Ashkenazi Jewish ancestry will emerge

TABLE 4____ Number of subjects Number withGrou
Jewish[a]Before age 37 40 4 (10)age 37-41 40 2 (5)Group 2Diagnosis ages 42-50 27 2
diagnosed with breast or ovarian cancer, one before age 50.

EXAMPLE 11 BRCA2 Shows a Low Somatic Mutation Rate in Breast Carcinoma and Other Cancers Including Ovarian and

BRCA2 is a tumor suppressor gene. A homozygous deletion of this gene may lead to breast cancer as well as other cance
heterozygosity or LOH). Mutations in both alleles may also lead to development of cancer. For studies here, an analysis of
mutations in BRCA2. Since compound mutant heterozygotes and mutant homozygotes are rare, tumor suppressor gene in

Identification of tumors and cell lines that exhibit LOH

A group of 104 primary breast tumor samples and a set of 269 cell lines was tested for LOH in the BRCA2 region. For prim
STRs:

(1) mM4247.4A.2F1 ACCATCAAACACATCATCC (SEQ ID NO: 119)

mM4247.4A.2R2 AGAAAGTAACTTGGAGGGAG (SEQ ID NO: 120)

(2) STR257-FC CTCCTGAAACTGTTCCCTTGG (SEQ ID NO: 121)

STR257-RD TAATGGTGCTGGGATATTTGG (SEQ ID NO: 122)

(3) mMB561A-3.1FA2 GAATGTCGAAGAGCTTGTC (SEQ ID NO: 123)

mMB561A-3.1RB AAACATACGCTTAGCCAGAC (SEQ ID NO: 124)

The PCR products were resolved using an ABI 377sequencer and quantified with Genescan software (ABI). For tumors, cl
tumor miscalled based on later analysis of single base polymorphisms. The heterozygosity indices for the markers are: ST
individual (assuming linkage equilibrium) is only one in 250. Due to the presence of normal cells in the primary tumor samp
heights from the tumor (FIGS. 5A-5D). Based on this analysis, 30 tumors (29%) were classified as having LOH at the BRC

LOH was assessed in the set of cell lines in a different fashion. Since homozygosity of all three STRs was improbable, and
particular tumor cell type under consideration. For example, 4/6 ovarian cell lines and 31/62 lung cancer lines displayed LO

Sequence Analysis of LOH Primary Breast Tumors and Cell Lines

The 30 primary breast cancers identified above which showed LOH in the BRCA2 region were screened by DNA sequence
radioactive mutation screen, the amplified products were purified by Qiagen beads (Qiagen, Inc.). DNA sequence was gen
electrophoresis on ABI 377sequencers. Samples were gridded into 96-well trays to facilitate PCR and sequencing. Dropou
were confirmed by sequencing a newly amplified PCR product to exclude the possibility that the sequence alteration was d

TABLE 5____Type # LOH/# Screened Percentage LOH
31% 5Lung 31/62 50% 20Lymphoma 0/4 0% 0Melanoma 17/81 21% 9Neuroblastor
30/104 29% 42____

LOH analysis of cell lines and primary breast tumors. Percentage LOH was calculated two ways: as total and as a mean of

Of the 30samples, two specimens contained frameshift mutations, one a nonsense mutation, and two contained missense
sequence variants were also present in the corresponding normal DNA from these cancer patients. To exclude the unlikely
Three of these revealed missense changes that were also found in the normal samples. Thus, in a set of 42 breast carcino
patients. The missense variants are rare; they were each observed only once during analysis of 115 chromosomes. From t

Of the 85 cell lines which displayed LOH (see Table 5), 58 were also screened for sequence changes. Greater than 95% o
BT111 primary tumor sample and to a previously detected germline frameshift (Tavtigian et al., 1996). This suggests that t

Detection of a probable germline BRCA2 mutation in a pancreatic tumor cell line suggests that BRCA2 mutations may pred
pancreatic xenograft (Schutte et al., 1995). Because only three pancreatic cell lines were examined in our study, further inv

TABLE 6A____Sample Type LOH Change Effect Germline_____
Frameshift yes4F8 Ovarian yes C2117T Thr→IleBT163 Primary breast no A2411C
Thr→MetBT111 Primary breast yes 6174delT Frameshift yes4G3 Panc
Gln→His yes3D5 Melanoma yes A9537G Ile→MetBT85 Primary breast yes A10204
yes____

Germline mutations identified in BRCA2. Listed are the mutation positions based on the Genbank entry of BRCA2 (Sehutte

TABLE 6B____Position Change Effect Frequency______________
A/G silent 0.35PM(3668) A/G Asn→Ser 0 (0.15)PM(4035) T/C silent 0.24 (0.10)PM(74
silent <0.01____

Common polymorphisms and silent substitutions detected in BRCA2 by DNA sequencing. Since some rare silent variants r
shown in parentheses (Tavtigian et al., 1996). Numbering is as in Table 6A.

Industrial Utility

As previously described above, the present invention provides materials and methods for use in testing BRCA2 alleles of a

protective effect of an early, full term pregnancy. Therefore, women at risk could consider early childbearing or a therapy de
be highly motivated to have regular mammograms, perhaps starting at an earlier age than the general population. Ovarian
precursor lesions. With the evolution of the method and the accumulation of information about BRCA2 and other causative

Women with breast cancers may follow different surgical procedures if they are predisposed, and therefore likely to have a
product. Alternatively, the therapeutic agent could be another molecule that mimics the deleterious gene's function, either a
counteract the effect of the deleterious allele. These gene therapies may take many forms and may be directed either towa

It will be appreciated that the methods and compositions of the instant invention can be incorporated in the form of a variet
be construed as restrictive.

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List of Patents and Patent Applications

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EPO Publication No. 225,807

European Patent Application Publication No. 0332435

Geysen, H., PCT published application WO 84/03564, published 13 Sep. 1984

Hitzeman et al., EP 73,675A

PCT published application WO 93/07282

____SEQUENCE LISTING(1) GE
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CHARACTERISTICS:(A) LENGTH: 33 base pairs(B) TYPE: nucleic acid(C) STRANDEDNESS: single(D) TOPOLOGY: line
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SENSE: NO(vi) ORIGINAL SOURCE:(A) ORGANISM: Homos sapiens(ix) FEATURE:(A) NAME/KEY: misc_ feature(B) LO
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CLASSIFICATIONS

U.S. Classification	435/69.1, 435/320.1, 435/375, 530/828
International Classification	A61K48/00, C07K14/47, C12N15/12
Cooperative Classification	Y10S530/828, A61K48/00, C07K14/4703
European Classification	C07K14/47A1A

LEGAL EVENTS

Date	Code	Event	
Apr 22, 2010	FPAY	Fee payment	Year of fee payment: 12
Apr 28, 2006	FPAY	Fee payment	Year of fee payment: 8
Apr 27, 2006	AS	Assignment	Owner name: UTAH RESEARCH FOUNDATION, UNI Free format text: ASSIGNMENT OF ASSIGNORS INT Effective date: 20060420
May 17, 2002	FPAY	Fee payment	Year of fee payment: 4
Oct 9, 1996	AS	Assignment	Owner name: ENDO RECHERCHE INC., CANADA Free format text: ASSIGNMENT OF ASSIGNORS INT Effective date: 19960819 Owner name: HOSPITAL FOR SICK CHILDREN, CAN Free format text: ASSIGNMENT OF ASSIGNORS INT Owner name: HSC RESEARCH & DEVELOPMENT L Free format text: ASSIGNMENT OF ASSIGNORS INT

Effective date: 19960617
Owner name: MYRIAD GENETICS, INC., UTAH
Free format text: ASSIGNMENT OF ASSIGNORS INT
Owner name: PENNSYLVANIA, THE TRUSTEES OF
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