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(54) Title: DIFFERENTIATION BETWEEN BRCA2-ASSOCIATED TUMOURS AND SPORADIC TUMOURS VIA ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

(57) Abstract: Array comparative genomic hybridization classifiers, arrays comprising the classifiers and methods of using the same for differentiating between BRCA2-associated tumors and sporadic tumors by detecting phenotypic genetic traits using comparative genomic hybridization are disclosed.

FIGURE 1A

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
2	RP11-560C7	RP5-960D23	2p24.1	2p16.3	22998568	47897750	24.9	
2	RP11-86O17	RP11-263G22	2q36.3	2q37.1	226629534	234701766	8.1	loss in SPOR
3	RP11-510B7	RP11-12A13	3p12.3	3q11.2	80611850	96250469	15.6	
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
6	GS-62-L11	RP11-199A24	6p25.3	6p11.1	135996	58788604	58.7	gain in BRCA2
6	RP11-349P19	RP11-256L9	6q12	6q13	65158448	73217550	8.1	gain in BRCA2
7	RP4-756H11	RP4-635O5	7q11.21	7q11.22	65647490	71274716	5.6	
7	RP4-811H12	GS-3-K23	7q35	7q36.3	146787501	158864424	12.1	
10	RP11-118K6	RP13-355A21	10p15.2	10p12.1	3035538	28141734	25.1	
10	RP11-90J7	RP11-436O19	10q22.3	10q26.13	79687950	124268654	44.6	loss in BRCA2
11	RP11-113A6	RP11-327O2	11p15.5	11p15.4	2261568	10454001	8.2	
11	RP11-569N5	RP11-137O10	11q13.2	11q14.2	68072319	87028461	19.0	
11	RP11-108O10	GS-26-N8	11q23.1	11q25	111095144	134437384	23.3	
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-468E2	RP11-168D12	14q12	14q21.2	23572376	41500293	17.9	
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-429K17	RP11-2C24	16p12.3	16p11.2	20151302	30747839	10.6	gain in SPOR
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP1-27I12	RP11-121A13	17p12	17p11.2	14285924	20230382	5.9	
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR
22	RP11-80O7	RP1-172B20	22q11.23	22q13.1	22510132	38559115	16.0	
23	GS-839-D20	RP11-576G22	23p22.33	23p11.3	110000	45268722	45.2	
23	RP5-965E19	RP5-1087L19	23q26.2	23q28	131271371	153547872	22.3	

WO 2011/048498 A2

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DIFFERENTIATION BETWEEN BRCA2-ASSOCIATED TUMOURS AND SPORADIC TUMOURS VIA ARRAY COMPARATIVE GENOMIC HYBRIDIZATION

Cross Reference to Related Applications

[001] This Patent Cooperation Treaty (PCT) patent application claims priority to United States provisional patent application No. 61/279,584, filed October 19, 2009, entitled "Methods for Differentiation Between BRCA2-Associated Tumours and Sporadic Tumours" the contents of which are incorporated herein by reference, in their entirety.

Field

[002] Array comparative genomic hybridization classifiers, arrays comprising the classifiers, and related methods provided by the present disclosure may be used to differentiate between BRCA2-associated tumours and sporadic tumors.

Background

[003] Breast cancer is the most common cancer in the developed countries and one of the leading causes of death in women; one out of every nine women will be affected by breast cancer. Approximately 10-15% of patients with breast cancer have a positive family history for breast cancer, and of those, approximately 25-50% is due to a mutation in the gene or genes that code for the breast cancer predisposition genes BRCA1 and/or BRCA2 (*see* Narod and Foulkes, 2004, Nat. Rev. Cancer. 4(9):665-76).

[004] BRCA2 (Breast Cancer Type 2 susceptibility protein) is a protein encoded by the BRCA2 gene. The BRCA2 gene is located on the long (q) arm of chromosome 13 at position 12.3 (13q12.3), from base pair 31,787,616 to base pair 31,871,804 (*see* Wooster *et al.*, 1994, Science 265(5181): 2088–90).

[005] BRCA2 belongs to the tumor suppressor gene family and is thought to be involved in the repair of chromosomal damage, specifically the repair of breaks in double-stranded DNA. BRCA2 thus helps maintain the stability of the human genome and helps prevent gene mutations and rearrangements that can lead to cancers.

[006] Mutations of the BRCA2 gene can cause the BRCA2 protein to be abnormal and defective. Defective BRCA2 protein is unable to function normally and thus cannot repair breaks in DNA. As a result, mutations build up that can cause uncontrolled cell growth, leading to cancers.

[007] In addition to breast cancer in men and women, mutations in the BRCA2 gene can lead to an increased risk of ovarian, fallopian, prostate, and pancreatic cancers, as well as

malignant melanoma. Several other types of cancer have also been seen in certain families carrying BRCA2 gene mutations.

[008] Identification of a mutation in the BRCA2 gene in a patient can assist a health care provider in determining the proper course of treatment for the patient. Additionally, mutation identification allows for pre-symptomatic mutation screening in family members.

[009] The current strategy to identify BRCA2 gene mutation carriers is to select eligible patients based on prediction models that use age and family history. Mutation screening is then performed. However, it is not clear to what extent BRCA2 mutation carriers are properly identified, as the cause of breast cancer in many families with a history of breast cancer remain unexplained. Prediction models are imperfect and are dependent on the number of family members from which information is available. Mutation screening may identify unclassified variants (UV) in the BRCA2 gene for which the pathogenicity is unknown, as the effect on BRCA2 protein function is unknown. Although functional assays for BRCA2 mutations exist, they are laborious, difficult to interpret in clinical terms, limited to only a number of protein functions, and thus not yet applicable in a diagnostic setting.

[010] For BRCA1-mutated tumors, several molecular portraits have been generated using copy number alterations and gene expression patterns, which can be used to successfully identify BRCA1-associated tumours. For BRCA2-mutated tumors, however, no specific genetic signature has been identified and the immunohistochemical phenotype is poorly defined. Although previous studies have investigated differences between BRCA1-mutated, BRCA2-mutated and sporadic breast tumors in gene expression patterns and copy number alterations, these molecular portraits have not been clinically validated or evaluated.

Summary

[011] Thus, a validated BRCA2 genetic signature, independent of tumor grade and receptor status, is useful.

[012] It is an object of the present disclosure to provide for a method and means for prognostic and/or diagnostic genomic profiling of tumours for BRCA2 involvement. Therefore, one goal of the present disclosure is to evaluate profiling of somatic genetic changes in breast tumors as a new strategy that can give additional information about the involvement of BRCA2 in tumorigenesis.

[013] In a first aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or in some embodiments a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-

12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28, and wherein a variation in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-associated tumor or a sporadic tumor. In some embodiments, the genomic copy number variations are detected at all 25 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[014] In a second aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or in some embodiments a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in one, or in some embodiments a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31, and wherein a variation in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-associated tumor or a sporadic tumor. In some embodiments, the genomic copy number variations are detected at all 7

genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, and greater than 6. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[015] In a third aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, are disclosed, wherein the classifier comprises at least one, or in some embodiments a plurality, of the BAC clones set forth in **Fig. 2**. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected using at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2**, and wherein a variation in copy number at any one or more of the BAC clones, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from either a BRCA2-associated tumor or a sporadic tumor. In some embodiments, the genomic copy number variations are detected using all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, the genomic copy number variations are detected using a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, the genomic copy number variations are detected using a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

[016] In a fourth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or in some embodiments a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or

in some embodiments a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1, and wherein an increase in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a BRCA2-associated tumor. In some embodiments, the genomic copy number variations are detected at all 3 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1 and greater than 2. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 3, and less than 2.

[017] In a fifth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or in some embodiments a plurality, of genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33, and wherein a decrease in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a BRCA2-associated tumor. In some embodiments, the genomic copy number variations are detected at all 3 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1 and greater than 2. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 3, and less than 2.

[018] In a sixth aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in the genomic locus 16p12.3-11.2 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are detected at the genomic locus 16p12.3-11.2, and wherein an increase in copy number at 16p12.3-11.2, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a sporadic tumor.

[019] In a seventh aspect, methods for using a BRCA2 aCGH classifier to detect genomic copy number variations in a test sample, as compared to a reference sample, in one, or in some embodiments a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 are disclosed. The methods comprise detecting genomic copy number variations in a test sample, wherein the copy number variations are

detected in at least one, or in some embodiments a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31, and wherein a decrease in copy number at any one or more of the genomic loci, as compared to the number of copies of DNA from a reference sample, classifies the cell sample as from a sporadic tumor. In some embodiments, the genomic copy number variations are detected at all 5 genomic loci. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from greater than 1, greater than 2, greater than 3, and greater than 4. In some embodiments, the genomic copy number variations are detected at a number of genomic loci selected from less than 5, less than 4, less than 3, and less than 2.

Brief Description of the Drawings

[020] Those skilled in the art will understand that the drawings, described herein, are for illustration purposes only. The drawings are not intended to limit the scope of the present disclosure.

[021] **Fig. 1A** depicts the BRCA2-associated genomic loci used to identify breast cancers with a BRCA2-deficient homologous recombination dependent DNA repair system.

[022] **Fig. 1B** depicts a subset of the BRCA2-associated genomic loci of **Fig. 1A**.

[023] **Fig. 2** depicts exemplary BAC clones that may be used to detect, or to generate probes to detect, copy number aberrations in the genomic loci of **Figs. 1A** and **1B**.

Detailed Description

Definitions

[024] "Array" refers to an arrangement, on a substrate surface, of multiple nucleic acid probes (as defined herein) of predetermined identity. In various embodiments, the sequences of each of the multiple nucleic acid probes are known. In general, an array comprises a plurality of target elements, each target element comprising one or more nucleic acid probes immobilized on one or more solid surfaces, to which sample nucleic acids can be hybridized. In various embodiments, each individual probe is immobilized to a designated, discrete location (*i.e.*, a defined location or assigned position) on the substrate surface. In various embodiments, each nucleic acid probe is immobilized to a discrete location on an array and each has a sequence that is either specific to, or characteristic of, a particular genomic locus. A nucleic acid probe is specific to, or characteristic of, a genomic locus when it contains a nucleic acid sequence that is unique to that genomic locus. Such a probe preferentially hybridizes to a nucleic acid made from that genomic locus, relative to nucleic acids made from other genomic loci.

[025] The nucleic acid probes can contain sequence(s) from specific genes or clones. In various embodiments, at least some of the nucleic acid probes contain sequences from any one or more of the specific genomic regions recited in **Fig. 1A**. In various embodiments, at least some of the nucleic acid probes contain sequences from any one or more of the specific genomic regions recited in **Fig. 1B**. In various embodiments, at least some of the nucleic acid probes contain sequences of known, reference genes or clones. In various embodiments, the nucleic acid probes in a single array contain both sequences from any one or more of the specific genomic regions recited in **Fig. 1A** and sequences of known, reference genes or clones. In various embodiments, the nucleic acid probes in a single array contain both sequences from any one or more of the specific genomic regions recited in **Fig. 1B** and sequences of known, reference genes or clones.

[026] The probes may be arranged on the substrate in a single density, or in varying densities. The density of each of the probes can be varied to accommodate certain factors such as, for example, the nature of the test sample, the nature of a label used during hybridization, the type of substrate used, and the like. Each probe may comprise a mixture of nucleic acids of varying lengths and, thus, varying sequences. For example, a single probe may contain more than one copy of a cloned nucleic acid, and each copy may be broken into fragments of different lengths. Each length will thus have a different sequence.

[027] The length, sequence and complexity of the nucleic acid probes may be varied. In various embodiments, the length, sequence and complexity are varied to provide optimum hybridization and signal production for a given hybridization procedure, and to provide the required resolution among different genes or genomic locations.

[028] "BRCA2 -associated tumor" means a tumor having cells containing a mutation of the BRCA2 locus or a deficiency in the homologous recombination-dependent double strand break DNA repair pathway that alters BRCA2 activity or function, either directly or indirectly.

[029] "CGH" or "Comparative Genomic Hybridization" refers generally to molecular-cytogenetic techniques for the analysis of copy number changes, gains and/or losses, in the DNA content of a given subject's DNA. CGH can be used to identify chromosomal alterations, such as unbalanced chromosomal changes, in any number of cells including, for example, cancer cells. In various embodiments, CGH is utilized to detect one or more chromosomal amplifications and/or deletions of regions between a test sample and a reference sample.

[030] "Chromosomal locus" refers to a specific, defined portion of a chromosome.

[031] "Genome" refers to all nucleic acid sequences, coding and non-coding, present in each cell type of a subject. The term also includes all naturally occurring or induced variation of these sequences that may be present in a mutant or disease variant of any cell type, including, for example, tumor cells. Genomic DNA and genomic nucleic acids are thus nucleic acids isolated from a nucleus of one or more cells, and include nucleic acids derived from, isolated from, amplified from, or cloned from genomic DNA, as well as synthetic versions of all or any part of a genome.

[032] For example, the human genome consists of approximately 3.0×10^9 base pairs of DNA organized into 46 distinct chromosomes. The genome of a normal human diploid somatic cell consists of 22 pairs of autosomes (chromosomes 1 to 22) and either chromosomes X and Y (male) or a pair of X chromosomes (female) for a total of 46 chromosomes. A genome of a cancer cell may contain variable numbers of each chromosome in addition to deletions, rearrangements and amplification of any sub-chromosomal region or DNA sequence.

[033] "Genomic locus" refers to a specific, defined portion of a genome.

[034] "HBOC tumors" refers to tumors from patients from Hereditary Breast and Ovarian Cancer families, who display a negative screen result for BRCA1 and/or BRCA2 mutation. Such patients have a family history that include at least two diagnoses for breast cancer and one diagnosis for ovarian cancer.

[035] "Hybridization" refers to the binding of two single stranded nucleic acids via complementary base pairing. Extensive guides to the hybridization of nucleic acids can be found in: Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes Part I, Ch. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993), Elsevier, N.Y.; and Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (3rd ed.) Vol. 1-3 (2001), Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y. The phrases "hybridizing specifically to", "specific hybridization", and "selectively hybridize to", refer to the preferential binding, duplexing, or hybridizing of a nucleic acid molecule to a particular probe under stringent conditions. The term "stringent conditions" refers to hybridization conditions under which a probe will hybridize preferentially to its target subsequence, and to a lesser extent, or not at all, to other sequences in a mixed population (*e.g.*, a DNA preparation from a tissue biopsy). "Stringent hybridization" and "stringent hybridization wash conditions" are sequence-dependent and are different under different environmental parameters.

[036] Generally, highly stringent hybridization and wash conditions are selected to be about 5° C lower than the thermal melting point (Tm) for a specific sequence at a defined ionic strength and pH. The Tm is the temperature at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the Tm for a particular probe. Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on an array is 42° C using standard hybridization solutions, with the hybridization being carried out overnight. An example of highly stringent wash conditions is a 0.15 M NaCl wash at 72° C for 15 minutes. An example of stringent wash conditions is a wash in 0.2X Standard Saline Citrate (SSC) buffer at 65° C for 15 minutes. An example of a medium stringency wash for a duplex of, for example, more than 100 nucleotides, is 1X SSC at 45° C for 15 minutes. An example of a low stringency wash for a duplex of, for example, more than 100 nucleotides, is 4X to 6X SSC at 40° C for 15 minutes.

[037] "Micro- array" refers to an array that is miniaturized so as to require microscopic examination for visual evaluation. In various embodiments, the arrays used in the methods of the present disclosure are micro-arrays.

[038] "Nucleic acid" refers to a deoxyribonucleotide or ribonucleotide in either single- or double-stranded form and includes all nucleic acids comprising naturally occurring nucleotide bases as well as nucleic acids containing any and/or all analogues of natural nucleotides. This term also includes nucleic acid analogues that are metabolized in a manner similar to naturally occurring nucleotides, but at rates that are improved for the purposes desired. This term also encompasses nucleic-acid-like structures with synthetic backbone analogues including, without limitation, phosphodiester, phosphorothioate, phosphorodithioate, methylphosphonate, phosphoramidate, alkyl phosphotriester, sulfamate, 3'-thioacetal, methylene(methylimino), 3'-N-carbamate, morpholino carbamate, and peptide nucleic acids (PNAs) (*see, e.g.*: "Oligonucleotides and Analogues, a Practical Approach," edited by F. Eckstein, IRL Press at Oxford University Press (1991); "Antisense Strategies," Annals of the New York Academy of Sciences, Volume 600, Eds. Baserga and Denhardt (NYAS 1992); Milligan (1993) J. Med. Chem. 36:1923-1937; and "Antisense Research and Applications" (1993, CRC Press)). PNAs contain non-ionic backbones, such as N-(2-aminoethyl) glycine units. Phosphorothioate linkages are described in: WO 97/03211; WO 96/39154; and Mata (1997) Toxicol. Appl. Pharmacol. 144:189-197. Other synthetic backbones encompassed by this term include methyl-phosphonate linkages or alternating

methyl-phosphonate and phosphodiester linkages (Strauss-Soukup (1997) Biochemistry 36: 8692-8698), and benzyl-phosphonate linkages (Samstag (1996) Antisense Nucleic Acid Drug Dev 6: 153-156).

[039] "Probe" or "nucleic acid probe" refer to one or more nucleic acid fragments whose specific hybridization to a sample can be detected. In various embodiments, probes are arranged on a substrate surface in an array. The probe may be unlabelled, or it may contain one or more labels so that its binding to a nucleic acid can be detected. In various embodiments, a probe can be produced from any source of nucleic acids from one or more particular, pre-selected portions of a chromosome including, without limitation, one or more clones, an isolated whole chromosome, an isolated chromosome fragment, or a collection of polymerase chain reaction (PCR) amplification products.

[040] In some embodiments, the probe may be a member of an array of nucleic acids as described in WO 96/17958. Techniques capable of producing high density arrays can also be used for this purpose (*see, e.g.*, Fodor (1991) Science 767-773; Johnston (1998) Curr. Biol. 8: Rl 71 -Rl 74; Schummer (1997) Biotechniques 23: 1087-1092; Kern (1997) Biotechniques 23: 120-124; and U.S. Patent No. 5,143,854).

[041] The sequence of the probes can be varied. In various embodiments, the probe sequence can be varied to produce probes that are substantially identical to the probes disclosed herein, but that retain the ability to hybridize specifically to the same targets or samples as the probe from which they were derived.

[042] "Reference sample" refers to nucleic acids comprising sequences whose quantity or degree of representation, copy number, and/or sequence identity are known. Such nucleic acids serve as a reference to which one or more test samples are compared.

[043] "Sample" refers to a material, or mixture of materials, containing one or more components of interest. Samples include, but are not limited to, material obtained from an organism and may be directly obtained from a source, such as from a biopsy or from a tumor, or indirectly obtained such as after culturing and/or processing.

[044] "Test sample" refers to nucleic acids comprising sequences whose quantity or degree of representation, copy number, and/or sequence identity are unknown. In various embodiments, the present disclosure is directed to the detection of the quantity or degree of representation, copy number, and/or sequence identity of one or more test samples.

[045] Reference is now made in detail to certain embodiments of arrays and methods. The disclosed embodiments are not intended to be limiting of the claims. To the contrary, the claims are intended to cover all alternatives, modifications, and equivalents.

Arrays, Micro-Arrays and Probes

[046] In various aspects, the present disclosure relates to the determination of copy number changes in the DNA content of a given test sample, as compared to one or more reference samples. In some embodiments, the copy number changes comprise gains or increases in the DNA content of a test sample. In some embodiments, the copy number changes comprise losses or decreases in the DNA content of a test sample. In some embodiments, the copy number changes comprise both gains or increases and losses or decreases in the DNA content of a test sample.

[047] Determination of copy number changes can be determined by hybridizations that are performed on a solid support. For example, probes that selectively hybridize to specific chromosomal regions can be spotted onto a surface. In various aspects, the spots of probes are placed in an ordered pattern, or array, and the pattern is recorded to facilitate correlation of results. Once an array is generated, one or more test samples can be hybridized to the array. In various aspects, arrays comprise a plurality of nucleic acid probes immobilized to discrete spots (*i.e.*, defined locations or assigned positions) on a substrate surface.

[048] Thus, in several aspects, copy number changes of genomic loci are analyzed in an array-based approach. In some embodiments, copy number changes of genomic loci are analyzed using comparative genomic hybridization. In some embodiments, copy number changes of genomic loci are analyzed using array-based comparative genomic hybridization.

[049] Any of a variety of arrays may be used. A number of arrays are commercially available for use from Vysis Corporation (Downers Grove, III), Spectral Genomics Inc. (Houston, TX), and Affymetrix Inc. (Santa Clara, CA). Arrays can also be custom made for one or more hybridizations.

[050] Methods of making and using arrays are well known in the art (*see, e.g.*, Kern *et al.*, Biotechniques (1997), 23:120-124; Schummer *et al.*, Biotechniques (1997), 23:1087-1092; Solinas-Toldo *et al.*, Genes, Chromosomes & Cancer (1997), 20: 399-407; Johnston, Curr. Biol. (1998), 8: R171-R174; Bowtell, Nature Gen. (1999), Supp. 21:25-32; Watson *et al.*, Biol. Psychiatry (1999), 45: 533-543; Freeman *et al.*, Biotechniques (2000), 29: 1042-1046 and 1048-1055; Lockhart *et al.*, Nature (2000), 405: 827-836; Cuzin, Transfus. Clin. Biol. (2001), 8:291-296; Zarrinkar *et al.*, Genome Res. (2001), 11: 1256-1261; Gabig *et al.*, Acta Biochim. Pol. (2001), 48: 615-622; and Cheung *et al.*, Nature (2001), 40: 953-958; *see also, e.g.*, U.S. Patent Nos. 5,143,854; 5,434,049; 5,556,752; 5,632,957; 5,700,637; 5,744,305; 5,770,456; 5,800,992; 5,807,522; 5,830,645; 5,856,174; 5,959,098; 5,965,452;

6,013,440; 6,022,963; 6,045,996; 6,048,695; 6,054,270; 6,258,606; 6,261,776; 6,277,489; 6,277,628; 6,365,349; 6,387,626; 6,458,584; 6,503,711; 6,516,276; 6,521,465; 6,558,907; 6,562,565; 6,576,424; 6,587,579; 6,589,726; 6,594,432; 6,599,693; 6,600,031; and 6,613,893).

[051] Substrate surfaces suitable for use in the generation of an array can be made of any rigid, semi-rigid or flexible material that allows for direct or indirect attachment (*i.e.*, immobilization) of nucleic acid probes to the substrate surface. Suitable materials include, without limitation, cellulose (*see, e.g.*, U.S. Patent No. 5,068,269), cellulose acetate (*see, e.g.*, U.S. Patent No. 6,048,457), nitrocellulose, glass (*see, e.g.*, U.S. Patent No. 5,843,767), quartz and/or other crystalline substrates such as gallium arsenide, silicones (*see, e.g.*, U.S. Patent No. 6,096,817), plastics and plastic copolymers (*see, e.g.*, U.S. Patent Nos. 4,355,153; 4,652,613; and 6,024,872), membranes and gels (*see, e.g.*, U.S. Patent No. 5,795,557), and paramagnetic or supramagnetic microparticles (*see, e.g.*, U.S. Patent No. 5,939,261). When fluorescence is to be detected, arrays comprising cyclo-olefin polymers may be used (*see, e.g.*, U.S. Patent No. 6,063,338). The presence of reactive functional chemical groups (such as, for example, hydroxyl, carboxyl, and amino groups) present on the surface of the substrate material can be used to directly or indirectly attach nucleic acid probes to the substrate surface.

[052] More than one copy of each nucleic acid probe may be spotted onto an array. For example, each nucleic acid probe may be spotted onto an array once, in duplicate, in triplicate, or more, depending on the desired application. Multiple spots of the same probe allows for assessment of the reproducibility of the results obtained.

[053] Related nucleic acid probes may also be grouped together, in probe elements, on an array. For example, a single probe element may include a plurality of spots of related nucleic acid probes, which are of different lengths but that comprise substantially the same sequence or that are derived from the sequence of a specific genomic locus. Alternatively, a single probe element may include a plurality of spots of related nucleic acid probes that are fragments of different lengths resulting from digestion of more than one copy of a cloned nucleic acid. An array may contain a plurality of probe elements and probe elements may be arranged on an array at different densities.

[054] Array-immobilized nucleic acid probes may be nucleic acids that contain sequences from genes (*e.g.*, from a genomic library) including, for example, sequences that collectively cover a substantially complete genome, or any one or more subsets of a genome. In various embodiments, the sequences of the nucleic acid probes on an array comprise those

for which comparative copy number information is desired. In some embodiments, to obtain DNA sequence copy number information across an entire genome, an array comprising nucleic acid probes covering a whole genome or a substantially complete genome is used. In some embodiments, at least one relevant genomic locus has been determined and is used in an array, such that there is no need for genome-wide hybridization. In some embodiments, a plurality of relevant genomic loci have been determined and are used in an array, such that there is no need for genome-wide hybridization. In some embodiments, the array comprises a plurality of specific nucleic acid probes that originate from a discrete set of genes or genomic loci and whose copy number, in association with the type of condition or tumor is to be tested, is known. Additionally, the array may comprise nucleic acid probes that will serve as positive or negative controls. In some embodiments, the array comprises a plurality of nucleic acid sequences derived from karyotypically normal genomes.

[055] The probes may be generated by any number of known techniques (*see, e.g.*, Tijssen, Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes Part I, Ch. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays" (1993), Elsevier, N.Y.; Sambrook *et al.*, Molecular Cloning: A Laboratory Manual (3rd ed.) Vol. 1-3 (2001), Cold Spring Harbor Laboratory, Cold Spring Harbor Press, N.Y.; Innis (Ed.) "PCR Strategies" (1995), Academic Press: New York, N.Y.; and Ausubel (Ed.), "Short Protocols in Molecular Biology" 5th Ed. (2002), John Wiley & Sons). Nucleic acid probes may be obtained and manipulated by cloning into various vehicles. They may be screened and re-cloned or amplified from any source of genomic DNA.

[056] Nucleic acid probes may also be obtained and manipulated by cloning into vehicles including, for example, recombinant viruses, cosmids, or plasmids. Nucleic acid probes may also be synthesized *in vitro* by chemical techniques (*see, e.g.*, Nucleic Acids Res. (1997), 25: 3440-3444; Blommers *et al.*, Biochemistry (1994), 33: 7886-7896; and Frenkel *et al.*, Free Radic. Biol. Med. (1995), 19: 373-380). Probes may vary in size from synthetic oligonucleotide probes and/or PCR-type amplification primers of a few base pairs in length to artificial chromosomes of more than 1 megabases in length. In various embodiments, probes comprise at least 10, at least 12, at least 15, at least 18, at least 20, at least 22, at least 30, at least 50 or at least 100 contiguous nucleotides of a sequence present in a BAC clone set forth in **Fig. 2**. In various embodiments, probes also comprise at least 10, at least 12, at least 15, at least 18, at least 20, at least 22, at least 30, at least 50 or at least 100 contiguous nucleotides of a sequence present in one or more reference samples. In some embodiments, probes

comprise a sequence that is unique in a genome. In some embodiments, probes comprise a sequence that is unique in the human genome.

[057] Probes may be obtained from any number of commercial sources. For instance, several P1 clones are available from the DuPont P1 library (*see, e.g.*, Shepard *et al.*, Proc. Natl. Acad. Sci. USA (1994), 92: 2629), and available commercially from Incyte Corporation (Wilmington, DE). Various libraries spanning entire chromosomes are available commercially from Clontech Laboratories, Inc. (Mountain View, CA), or from the Los Alamos National Laboratory (Los Alamos, CA). In various aspects, the present disclosure relates to the use of the human 3600 BAC/PAC genomic clone set, covering the full human genome at 1 Mb spacing, obtained from the Wellcome Trust Sanger Institute (Hinxton, Cambridge, UK).

[058] In some embodiments, the nucleic acid probes are derived from mammalian artificial chromosomes (MACs) and/or human artificial chromosomes (HACs), which can contain inserts from about 5 to 400 kilobases (kb) (*see, e.g.*, Roush, Science (1997), 276: 38-39; Rosenfeld, Nat. Genet. (1997), 15: 333-335; Ascenzioni *et al.*, Cancer Lett. (1997), 118: 135-142; Kuroiwa *et al.*, Nat. Biotechnol. (2000), 18: 1086-1090; Meija *et al.*, Am. J. Hum. Genet. (2001), 69: 315-326; and Auriche *et al.*, EMBO Rep. (2001), 2: 102-107).

[059] In some embodiments, the nucleic acid probes are derived from satellite artificial chromosomes or satellite DNA-based artificial chromosomes (SATACs). SATACs can be produced by inducing *de novo* chromosome formation in cells of varying mammalian species (*see, e.g.*, Warburton *et al.*, Nature (1997), 386: 553-555; Csonka *et al.*, J. Cell. Sci. (2000), 113: 3207-3216; and Hadlaczky, Curr. Opin. Mol. Ther. (2001), 3: 125-132).

[060] In some embodiments, the nucleic acid probes are derived from yeast artificial chromosomes (YACs), 0.2-1 megabses in size. YACs have been used for many years for the stable propagation of genomic fragments of up to one million base pairs in size (*see, e.g.*, Feingold *et al.*, Proc. Natl. Acad. Sci. USA (1990), 87:8637-8641; Adam *et al.*, Plant J. (1997), 11: 1349-1358; Tucker *et al.*, Gene (1997), 199: 25-30; and Zeschnigk *et al.*, Nucleic Acids Res. (1999), 27: E30).

[061] In some embodiments, the nucleic acid probes are derived from bacterial artificial chromosomes (BACs) up to 300 kb in size. BACs are based on the E. coli F factor plasmid system and are typically easy to manipulate and purify in microgram quantities (*see, e.g.*, Asakawa *et al.*, Gene (1997), 191: 69-79; and Cao *et al.*, Genome Res. (1999), 9: 763-774).

[062] In some embodiments, the nucleic acid probes are derived from P1 artificial chromosomes (PACs), about 70-100 kb in size. PACs are bacteriophage Pl -derived vectors (*see, e.g.*, Ioannou *et al.*, Nature Genet. (1994), 6: 84-89; Boren *et al.*, Genome Res. (1996), 6: 1123-1130; Nothwang *et al.*, Genomics (1997), 41: 370-378; Reid *et al.*, Genomics (1997), 43: 366-375; and Woon *et al.*, Genomics (1998), 50: 306-316).

[063] In some embodiments, the array comprises a series of separate wells or chambers on the substrate surface, into which probes may be immobilized as described herein. The probes can be immobilized in the separate wells or chambers and hybridization can take place within the wells or chambers. In various embodiments, the arrays can be selected from chips, microfluidic chips, microtiter plates, Petri dishes, and centrifuge tubes. Robotic equipment has been developed for these types of arrays that permit automated delivery of reagents into the separate wells or chambers which allow the amount of the reagents used per hybridization to be sharply reduced. Examples of chip and microfluidic chip techniques can be found, for example, in U.S. Patent No. 5,800,690; Orchid, "Running on Parallel Lines" New Scientist (1997); McCormick *et al.*, Anal. Chem. (1997), 69:2626-30; and Turgeon, "The Lab of the Future on CD-ROM?" Medical Laboratory Management Report. December 1997, p. 1.

[064] In some embodiments, arrays may be generated by isolating DNA from one or more artificial chromosomes, such as for example BACs, according to standard procedures. For example, in some embodiments, DNA can be isolated from one or more BACs using a Qiawell plasmid kit (Qiagen, Chatsworth, CA). Total DNA can be amplified from the insert sites of the BACs via degenerate oligonucleotide primed PCR using a set of degenerate primers with a C6-NH_2 modification at their 5' end for covalent attachment to a substrate surface. The substrates may be any type suitable for such use including, for example, CODELINK™ glass slides (Corning, Cambridge, UK). Covalent attachment to the substrate can occur via the manufacturer's suggested protocols, or via other detailed protocols (such as those described in Pinkel *et al.*, Nature Genetics (1998), 20:207-211) with some modifications (such as those described in Alers et al. 1999). The DNA obtained after PCR amplification can then be spotted onto the substrate surface for covalent attachment thereto. The DNA may be spotted as a single site, in duplicate or in triplicate on the substrate surface.

BRCA2 Arrays

[065] In various aspects, the present disclosure relates to the use of a BRCA2 array to identify breast cancers with a deficient homologous recombination-dependent double strand break DNA repair system due to BRCA2 dysfunction and to thus distinguish BRCA2-

associated tumors from sporadic tumors. Therefore, in various aspects, the present disclosure relates to the use of a BRCA2 array comprising a unique BRCA2 aCGH profile to distinguish BRCA2-associated tumors from sporadic tumors by detecting phenotypic genetic traits associated with deficiencies in the BRCA2 gene. In further aspects, the present disclosure relates to the use of a BRCA2 array comprising a unique BRCA2 aCGH profile to distinguish BRCA2-associated tumors from sporadic tumors by detecting phenotypic genetic traits associated with deficiencies in non-BRCA2 genes, wherein the deficiencies negatively affect the homologous recombination-dependent double strand break DNA repair pathway of which BRCA2 is a component.

[066] In various embodiments, a BRCA2 array comprising a BRCA2 aCGH profile for distinguishing BRCA2-associated tumors from sporadic tumors, is provided. In various aspects, arrays provided by the present disclosure, which in some embodiments are BRCA2 arrays, can comprise at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2** immobilized on a substrate surface. In various aspects, arrays provided by the present disclosure, which in some embodiments are BRCA2 arrays, can comprise at least one, or in some embodiments a plurality, of the BAC clones of **Fig. 2** immobilized to discrete spots on a substrate surface. In some embodiments, an array comprises all 704 of the BAC clones set forth in **Fig. 2** immobilized on a substrate surface. In some embodiments, an array comprises all 704 of the BAC clones set forth in **Fig. 2**, immobilized to a plurality of discrete spots on a substrate surface. In some embodiments, arrays provided by the present disclosure comprise a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, the BAC clones comprising the arrays of the preceding sentence are immobilized to a plurality of discrete spots on a substrate surface. In some embodiments, arrays provided by the present disclosure comprise a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100,

less than 75, les than 50, less than 25, less than 20, and less than 10. In some embodiments, the BAC clones comprising the arrays of the preceding sentence are immobilized to a plurality of discrete spots on a substrate surface. In various aspects, arrays provided by the present disclosure can also comprise at least one, or in some embodiments a plurality, of nucleic acid probes from a reference sample immobilized on a substrate surface. In various aspects, arrays provided by the present disclosure can also comprise at least one, or in some embodiments a plurality, of nucleic acid probes from a reference sample immobilized to discrete spots on a substrate surface. In some embodiments, a BRCA2 array is used to detect BRCA2-associated genomic copy number variations in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array is used to detect BRCA2-associated genomic copy number variations in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

[067] In some embodiments, a BRCA2 array is used to detect an increase in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array is used to detect a decrease in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In the aforementioned embodiments, detection of genomic copy number variations in the test sample, as compared to the reference sample, classifies the test sample as from a BRCA2-associated tumor.

[068] In some embodiments, a BRCA2 array is used to detect an increase in genomic copy numbers in a test sample, as compared to a reference sample, at the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array is used to detect a decrease in genomic copy numbers in a test sample, as compared to a reference sample, at one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In the aforementioned embodiments, detection of genomic copy number variations in the test sample, as compared to the reference sample, classifies the test sample as from a sporadic tumor.

[069] The genomic loci may be detected individually, or in any combination of two or more loci. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in all 25 of the above-listed chromosomal loci. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations at a number of the above-listed genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations at a number of the above-listed genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in all 25 of the BRCA2-associated genomic loci set forth in **Fig. 1A**. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in all 7 of the BRCA2-associated genomic loci set forth in **Fig. 1B**. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In some embodiments, a BRCA2 array is used that is

capable of detecting BRCA2-associated genomic copy number variations in at the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array is used that is capable of detecting BRCA2-associated genomic copy number variations in at least one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In each of the aforementioned embodiments, detection of BRCA2-associated genomic copy number variations classifies the test sample as from either a BRCA2-associated tumor or from a sporadic tumor.

[070] The BRCA2 arrays comprise at least one probe. In various embodiments, the BRCA2 arrays comprise a plurality of probes. In some embodiments, the BRCA2 arrays comprise a plurality of probes, wherein the probes comprise nucleic acid sequences derived from BAC clones. The BRCA2-associated genomic loci set forth in **Fig. 1A** are bounded by the BAC probes set forth in **Fig 2**. The BRCA2-associated genomic loci set forth in **Fig. 1B** are bounded by a sub-set of the BAC probes set forth in **Fig 2**. In some embodiments, arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of probes derived from the BAC clones of **Fig. 2**. The BAC clones set forth in **Fig. 2** are not intended to be limiting in any way, and other probes within the BRCA2-associated genomic loci of **Figs. 1A** and **1B** can also be used in the BRCA2 arrays. In some embodiments, arrays capable of detecting BRCA2-associated genomic copy number variations comprise all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, arrays capable of detecting BRCA2-associated genomic copy number variations comprise a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, arrays capable of detecting BRCA2-associated genomic copy number variations comprise a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

[071] In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to the genomic locus 16p12.3-11.2. In some embodiments, a BRCA2 array capable of detecting BRCA2-associated genomic copy number variations comprises at least one, or a plurality, of probes that independently hybridize to a genomic locus selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. In these embodiments, the number of probes used can be determined as described above, the probes are as defined above and/or the probes may be obtained in methods as described above.

[072] In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise at least one, or a plurality of the distinct BAC clones of **Fig. 2**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality of probes, wherein the probes comprise at least one, or a plurality, of the BAC clones of **Fig. 2**, and wherein the probes specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of

the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise a plurality of probes, wherein the nucleic acid sequences of the probes are unique to the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise a plurality of probes, wherein the probes comprise a plurality of BAC clones specific to all of the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality of probes, wherein the probes comprise at least one, or a plurality, of the BAC clones of **Fig. 2**, and wherein the probes specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise a plurality of probes, wherein the nucleic acid sequences of the probes are unique to the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise a plurality of probes, wherein the probes comprise a plurality of BAC clones specific to all of the genomic loci set forth in **Fig. 1B**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 15, at least 20, at least 30, at least 50, at least 60, at least 80 or at least 100 of the distinct BAC clones of **Fig. 2**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least three probes, wherein the probes comprise greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, or greater than 700 distinct BAC clones of **Fig. 2** that specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of the genomic loci set forth in **Fig. 1A**. In some embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of probes, wherein the probes comprise greater

than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, or greater than 700 distinct BAC clones of **Fig. 2** that specifically hybridize to at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**.

[073] In various embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations that comprise at least one, or a plurality, of probes, and/or that comprise at least one, or a plurality, of distinct BAC clones, allow for the individual analysis of at least one, or a plurality, of distinct genomic loci. Therefore, in some embodiments, the probes, and/or the distinct BAC clones, capable of detecting BRCA2-associated genomic copy number variations are arranged on the BRCA2 arrays in a positionally-addressable manner.

[074] In various embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24 or at least 25 of the genomic loci set forth in **Fig. 1A**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent at least 1, at least 2, at least 3, at least 4, at least 5, at least 6 or at least 7 of the genomic loci set forth in **Fig. 1B**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent all 25 of the genomic loci set forth in **Fig. 1A**. In various embodiments, BRCA2 arrays capable of detecting BRCA2-associated genomic copy number variations comprise at least one, or a plurality, of distinct BAC clones, wherein the distinct BAC clones represent all 7 of the genomic loci set forth in **Fig. 1B**.

Array Comparative Genomic Hybridization

[075] In various aspects, the present disclosure relates to the analysis of tumor cell samples by array-based comparative genomic hybridization. Array comparative genomic

hybridization (aCGH) is a technique that is used to detect genomic copy number variations at a higher level of resolution than chromosome-based comparative genomic hybridization. In aCGH, nucleic acids from a test sample and nucleic acids from a reference sample are labelled differentially. The test sample and the reference sample are then hybridized to an array comprising a plurality of probes. The ratio of the signal intensity of the test sample to that of the reference sample is then calculated, to measure the copy number changes for a particular location in the genome. The difference in the signal ratio determines whether the total copy numbers of the nucleic acids in the test sample are increased or decreased as compared to the reference sample. The test sample and the reference sample may be hybridized to the array separately or they may be mixed together and hybridized simultaneously. Exemplary methods of performing aCGH can be found, for example, in U.S. Patent Nos. 5,635,351; 5,665,549; 5,721,098; 5,830,645; 5,856,097; 5,965,362; 5,976,790; 6,159,685; 6,197,501; and 6,335,167; European Patent Nos. EP 1 134 293 and EP 1 026 260; van Beers *et al.*, Brit. J. Cancer (2006), 20; Joosse *et al.*, BMC Cancer (2007), 7:43; Pinkel *et al.*, Nat. Genet. (1998), 20: 207-211; Pollack *et al.*, Nat. Genet. (1999), 23: 41-46; and Cooper, Breast Cancer Res. (2001), 3: 158-175.

[076] Samples that are labelled differentially are labelled such that one of the two samples is labelled with a first detectable agent and the other of the two samples is labelled with a second detectable agent, wherein the first detectable agent and the second detectable agent produce distinguishable signals. Detectable agents that produce distinguishable signals can include, for example, matched pairs of fluorescent dyes.

[077] In some embodiments, the methods of the present disclosure comprise analyzing at least one test sample of tumor DNA from a subject by array-based comparative genomic hybridization to obtain information relating to the copy number aberrations present in the sample(s), if any; and, based on the information obtained, classifying the tumor as a BRCA2-related tumor, a BRCAlikeness tumor or a sporadic tumor.

[078] Information relating to the copy number aberrations present in a sample can include, for example, a gain of genetic material at one or more genomic loci, a loss of genetic material at one or more genomic loci, chromosomal abnormalities at one or more genomic loci, and genome copy number changes at one or more genomic loci. This information is obtained by analyzing the difference in signal intensity between the test sample and a reference sample at one or more genomic loci. The analysis can be performed using any of a variety of methods, means and variations thereof for carrying out array-based comparative genomic hybridization.

[079] In various embodiments, the reference sample is a nucleic acid sample that is representative of a normal, non-diseased state, for example a non-tumor/non-cancer cell, and contains a normal amount of copy numbers of the complement of the genomic loci being tested. The reference sample may be derived from a genomic nucleic acid sample from a normal and/or healthy individual or from a pool of such individuals. In various embodiments, the reference sample does not comprise any tumor or cancerous nucleic acids. In some embodiments, the reference sample is derived from a pool of female subjects. In some embodiments, the reference sample comprises pooled genomic DNA isolated from tissue samples (*e.g.* lymphocytes) from a plurality (*e.g.* at least 4-10) of healthy female subjects. In some embodiments, the reference sample comprises an artificially-generated population of nucleic acids designed to approximate the copy number level from each tested genomic region, or fragments of each tested genomic region. In some embodiments, the reference sample is derived from normal, non-cancerous cell lines or from cell line samples.

[080] Test samples may be obtained from a biological source comprising tumor cells, and reference samples may be obtained from a biological source comprising normal reference cells, by any suitable method of nucleic acid isolation and/or extraction. In various aspects, the test sample and the reference sample are DNA. Methods of DNA extraction are well known in the art. A classical DNA isolation protocol is based on extraction using organic solvents, such as a mixture of phenol and chloroform, followed by precipitation with ethanol (*see, e.g.*, Sambrook *et al.*, *supra*). Other methods include salting out DNA extraction, trimethylammonium bromide salt extraction, and guanidinium thiocyanate extraction. Additionally, there are numerous DNA extraction kits that are commercially available from, for example, BD Biosciences Clontech (Palo Alto, CA), Epicentre Technologies (Madison, WI), Gentra Systems, Inc. (Minneapolis, MN), MicroProbe Corp. (Bothell, WA), Organon Teknika (Durham, NC), and Qiagen Inc. (Valencia, CA).

[081] The test samples and the reference samples may be differentially labelled with any detectable agents or moieties. In various embodiments, the detectable agents or moieties are selected such that they generate signals that can be readily measured and such that the intensity of the signals is proportional to the amount of labelled nucleic acids present in the sample. In various embodiments, the detectable agents or moieties are selected such that they generate localized signals, thereby allowing resolution of the signals from each spot on an array.

[082] Methods for labeling nucleic acids are well-known in the art. For exemplary reviews of labeling protocols, label detection techniques and recent developments in the field,

see: Kricka, Ann. Clin. Biochem. (2002), 39: 114-129; van Gijlswijk *et al.*, Expert Rev. Mol. Diagn. (2001), 1: 81-91; and Joos *et al.*, J. Biotechnol. (1994), 35: 135-153. Standard nucleic acid labeling methods include: incorporation of radioactive agents, direct attachment of fluorescent dyes or of enzymes, chemical modification of nucleic acids to make them detectable immunochemically or by other affinity reactions, and enzyme-mediated labeling methods including, without limitation, random priming, nick translation, PCR and tailing with terminal transferase. Other suitable labeling methods include psoralen-biotin, photoreactive azido derivatives, and DNA alkylating agents. In various embodiments, test sample and reference sample nucleic acids are labelled by Universal Linkage System, which is based on the reaction of monoreactive cisplatin derivatives with the N7 position of guanine moieties in DNA (*see, e.g.*, Heetebrij *et al.*, Cytogenet. Cell. Genet. (1999), 87: 47-52).

[083] Any of a wide variety of detectable agents or moieties can be used to label test and/or reference samples. Suitable detectable agents or moieties include, but are not limited to: various ligands; radionuclides such as, for example, ^{32}P, ^{35}S, ^{3}H, ^{14}C, ^{125}I, ^{131}I, and others; fluorescent dyes; chemiluminescent agents such as, for example, acridinium esters, stabilized dioxetanes, and others; microparticles such as, for example, quantum dots, nanocrystals, phosphors and others; enzymes such as, for example, those used in an ELISA, horseradish peroxidase, beta-galactosidase, luciferase, alkaline phosphatase and others; colorimetric labels such as, for example, dyes, colloidal gold and others; magnetic labels such as, for example, Dynabeads™; and biotin, dioxigenin or other haptens and proteins for which antisera or monoclonal antibodies are available.

[084] In some embodiments, the test samples and the reference samples are labelled with fluorescent dyes. Suitable fluorescent dyes include, without limitation, Cy-3, Cy-5, Texas red, FITC, Spectrum Red, Spectrum Green, phycoerythrin, rhodamine, and fluorescein, as well as equivalents, analogues and/or derivatives thereof. In some embodiments, the fluorescent dyes selected display a high molar absorption coefficient, high fluorescence quantum yield, and photostability. In some embodiments, the fluorescent dyes exhibit absorption and emission wavelengths in the visible spectrum (i.e., between 400nm and 750nm) rather than in the ultraviolet range of the spectrum (i.e., lower than 400nm). In some embodiments, the fluorescent dyes are Cy-3 (3-N,N'-diethyltetramethylindo-dicarbocyanine) and Cy-5 (5-N,N'-diethyltetramethylindo-dicarbocyanine). Cy-3 and Cy-5 form a matched pair of fluorescent labels that are compatible with most fluorescence detection systems for array-based instruments. In some embodiments, the fluorescent dyes are Spectrum Red and Spectrum Green.

[085] A key component of aCGH is the hybridization of a test sample and a reference sample to an array. Exemplary hybridization and wash protocols are described, for example, in Sambrook *et al.* (2001), *supra*; Tijssen (1993), *supra*; and Anderson (Ed.), "Nucleic Acid Hybridization" (1999), Springer Verlag: New York, N.Y. In some embodiments, the hybridization protocols used for aCGH are those of Pinkel *et al.*, Nature Genetics (1998), 20:207-211. In some embodiments, the hybridization protocols used for aCGH are those of Kallioniemi, Proc. Natl. Acad. Sci. USA (1992), 89:5321-5325.

[086] Methods of optimizing hybridization conditions are well known in the art (*see, e.g.*, Tijssen, (1993), *supra*). To create competitive hybridization conditions, the array may be contacted simultaneously with differentially labelled nucleic acid fragments of the test sample and the reference sample. This may be done by, for example, mixing the labelled test sample and the labelled reference sample together to form a hybridization mixture, and contacting the array with the mixture.

[087] The specificity of hybridization may be enhanced by inhibiting repetitive sequences. In some embodiments, repetitive sequences (*e.g.*, Alu sequences, L1 sequences, satellite sequences, MRE sequences, simple homo-nucleotide tracts, and/or simple oligonucleotide tracts) present in the nucleic acids of the test sample, reference sample and/or probes are either removed, or their hybridization capacity is disabled. Removing repetitive sequences or disabling their hybridization capacity can be accomplished using any of a variety of well-known methods. These methods include, but are not limited to, removing repetitive sequences by hybridization to specific nucleic acid sequences immobilized to a solid support (*see, e.g.*, Brison *et al.*, Mol. Cell. Biol. (1982), 2: 578- 587); suppressing the production of repetitive sequences by PCR amplification using adequately designed PCR primers; inhibiting the hybridization capacity of highly repeated sequences by self-reassociation (*see, e.g.*, Britten *et al.*, Methods of Enzymology (1974), 29: 363-418); or removing repetitive sequences using hydroxyapatite which is commercially available from a number of sources including, for example, Bio-Rad Laboratories, Richmond, VA. In some embodiments, the hybridization capacity of highly repeated sequences in a test sample and/or in a reference sample is competitively inhibited by including, in the hybridization mixture, unlabelled blocking nucleic acids. The unlabelled blocking nucleic acids are therefore mixed with the hybridization mixture, and thus with a test sample and a reference sample, before the mixture is contacted with an array. The unlabelled blocking nucleic acids act as a competitor for the highly repeated sequences and bind to them before the hybridization mixture is contacted with an array. Therefore, the unlabelled blocking nucleic acids prevent labelled

repetitive sequences from binding to any highly repetitive sequences of the nucleic acid probes, thus decreasing the amount of background signal present in a given hybridization. In some embodiments, the unlabelled blocking nucleic acids are Human Cot-1 DNA. Human Cot-1 DNA is commercially available from a number of sources including, for example, Gibco/BRL Life Technologies (Gaithersburg, MD).

[088] Once hybridization is complete, the ratio of the signal intensity of the test sample as compared to the signal intensity of the reference sample is calculated. This calculation quantifies the amount of copy number aberrations present in the genomic DNA of the test sample, if any. In some embodiments, this calculation is carried out quantitatively or semi-quantitatively. In several aspects, it is not necessary to determine the exact copy number aberrations present in the genomic loci tested, as detection of an aberration, *i.e.* a gain or loss of genetic material, from the copy number in normal, non-cancerous genomic DNA is indicative of the presence of a disease state and is thus sufficient. Therefore, in several embodiments the quantification of the amount of copy number aberrations present in the genomic DNA of a test sample comprises an estimation of the copy number aberrations, as a semi-quantitative or relative measure usually suffices to predict the presence of a disease state and thus prospectively direct the determination of therapy for a subject.

[089] Quantitative techniques may be used to determine the copy number aberrations per cell present in a test sample. Several quantitative and semi-quantitative techniques to determine copy number aberrations exist including, for example, semi-quantitative PCR analysis or quantitative real-time PCR. The Polymerase Chain Reaction (PCR) *per se* is not a quantitative technique, however PCR-based methods have been developed that are quantitative or semi-quantitative in that they give a reasonable estimate of original copy numbers, within certain limits. Examples of such PCR techniques include, for example, quantitative PCR and quantitative real-time PCR (also known as RT-PCR, RQ-PCR, QRT-PCR or RTQ-PCR). In addition, many techniques exist that give estimates of relative copy numbers, as calculated relative to a reference. Such techniques include many array-based techniques. Absolute copy number estimates may be obtained by *in situ* hybridization techniques such as, for example, fluorescence *in situ* hybridization or chromogenic *in situ* hybridization.

[090] Fluorescence *in situ* hybridization permits the analysis of copy numbers of individual genomic locations and can be used to study copy numbers of individual genetic loci or particular regions on a chromosome (*see, e.g.*, Pinkel et al., Proc. Natl. Acad. Sci. U.S.A. (1988), 85, 9138-42). Comparative genomic hybridization can also be used to probe

for copy number changes of chromosomal regions (*see, e.g.*, Kallioniemi *et al.*, Science (1992), 258: 818-21; and Houldsworth *et al.*, Am. J. Pathol. (1994), 145: 1253-60).

[091] Copy numbers of genomic locations may also be determined using quantitative PCR techniques such as real-time PCR (*see, e.g.*, Suzuki *et al.*, Cancer Res. (2000), 60:5405-9). For example, quantitative microsatellite analysis can be performed for rapid measurement of relative DNA sequence copy numbers. In quantitative microsatellite analysis, the copy numbers of a test sample relative to a reference sample is assessed using quantitative, real-time PCR amplification of loci carrying simple sequence repeats. Simple sequence repeats are used because of the large numbers that have been precisely mapped in numerous organisms. Exemplary protocols for quantitative PCR are provided in Innis *et al.*, PCR Protocols, A Guide to Methods and Applications (1990), Academic Press, Inc. N.Y. Semi -quantitative techniques that may be used to determine specific DNA copy numbers include, for example, multiplex ligation-dependent probe amplification (*see, e.g.*, Schouten *et al.* Nucleic Acids Res. (2002), 30(12):e57; and Sellner *et al.*, Human Mutation (2004), 23(5):413-419) and multiplex amplification and probe hybridization (*see, e.g.*, Sellner *et al.* (2004), *supra*).

BRCA2 Array Comparative Genomic Hybridization

[092] In various aspects, the present disclosure relates to the use of a BRCA2 aCGH classifier capable of identifying BRCA2-associated tumors. In various aspects, a BRCA2 aCGH classifier capable of identifying BRCA2-associated tumors is set forth on a BRCA2 array, as described herein.

[093] Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 4p13-12, 13q12.2-21.1, 13q31.3-33.1, 14q23.2-32.33, 16q12.1-

21, 17q11.1-12 and 17q21.2-21.31. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in the genomic locus 16p12.3-11.2. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci set forth in **Fig. 1A**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, wherein the copy number variations are detected in at least one, or a plurality, of the genomic loci set forth in **Fig. 1B**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations at a number of the above-listed genomic loci selected from greater than 1, greater than 2, greater than 3, greater than 4, greater than 5, greater than 6, greater than 7, greater than 8, greater than 9, greater than 10, greater than 11, greater than 12, greater than 13, greater than 14, greater than 15, greater than 16, greater than 17, greater than 18, greater than 19, greater than 20, greater than 21, greater than 22, greater than 23, and greater than 24. In some embodiments, a BRCA2 aCGH classifier, which in

some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations at a number of the above-listed genomic loci selected from less than 25, less than 24, less than 23, less than 22, less than 21, less than 20, less than 19, less than 18, less than 17, less than 16, less than 15, less than 14, less than 13, less than 12, less than 11, less than 10, less than 9, less than 8, less than 7, less than 6, less than 5, less than 4, less than 3, and less than 2.

[094] Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample using at least one, or a plurality, of probes that independently hybridize to at least one, or a plurality, of the genomic loci set forth in **Fig. 1A**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample using at least one, or a plurality, of probes that independently hybridize to at least one, or a plurality, of the genomic loci set forth in **Fig. 1B**. Using the methods described above, in various aspects, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, is capable of detecting genomic copy number variations in a test sample, as compared to a reference sample, using at least one, or a plurality, of the distinct BAC clones set forth in **Fig. 2**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises all 704 of the BAC clones set forth in **Fig. 2**. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises a number of the BAC clones set forth in **Fig. 2** selected from greater than 1, greater than 10, greater than 20, greater than 25, greater than 50, greater than 75, greater than 100, greater than 125, greater than 150, greater than 175, greater than 200, greater than 225, greater than 250, greater than 275, greater than 300, greater than 325, greater than 350, greater than 375, greater than 400, greater than 425, greater than 450, greater than 475, greater than 500, greater than 525, greater than 550, greater than 575, greater than 600, greater than 625, greater than 650, greater than 675, and greater than 700. In some embodiments, a BRCA2 aCGH classifier, which in some embodiments is present in an array as described herein, capable of detecting genomic copy number variations in a test sample comprises a number of the BAC clones set forth in **Fig. 2** selected from less than 704, less than 700, less than 675, less than 650, less than 625, less than 600, less than 575, less than 550, less than 525, less

than 500, less than 475, less than 450, less than 425, less than 400, less than 375, less than 350, less than 325, less than 300, less than 275, less than 250, less than 225, less than 200, less than 175, less than 150, less than 125, less than 100, less than 75, les than 50, less than 25, less than 20, and less than 10.

Therapeutic Uses

[095] The present disclosure sets forth BRCA2 classifiers, which in some embodiments are present in one or more arrays as described herein, suitable for use in methods for distinguishing BRCA2-associated tumours from sporadic tumours. In various aspects, the BRCA2 classifiers can be used to distinguish between a cell sample from a BRCA2-associated tumor and a cell sample from a sporadic tumor. Using the methods described above, in various aspects, the BRCA2 classifiers are capable of determining whether an individual subject has a BRCA2-associated tumor. Using the methods described above, in various aspects, the BRCA2 classifiers are capable of determining whether an individual subject has a sporadic tumor. The BRCA2 classifiers are therefore capable of distinguishing between BRCA2-associated tumors and sporadic tumors.

[096] The BRCA2 classifiers can be used to evaluate somatic genetic changes in tumors to give additional information about the involvement of BRCA2 in tumorigenesis. The BRCA2 classifiers are capable of identifying BRCA2-associated tumors based on their genomic signature. As shown in the Examples, in some embodiments the BRCA2 classifiers are able to classify BRCA2-mutated tumors with a sensitivity of about 89% and a specificity of about 84%. The BRCA2 classifiers can thus be used as pre-selection tools, to prospectively detect subjects with a high risk of carrying a BRCA2 mutation. Additionally, the BRCA2 classifiers can be used as tests to identify breast cancer patients having BRCA2-associated tumors.

[097] As shown in the Examples, the BRCA2 classifiers can be used to investigate the chromosomal aberrations of BRCA2-mutated tumors to identify their molecular signature. In some embodiments, the BRCA2 classifiers can be used to distinguish BRCA2-associated tumors from sporadic tumors with about 86.5% accuracy. The BRCA2 classifiers can therefore be used to give additional indications about the involvement of BRCA2 in tumorigenesis of tumors where the role of BRCA2 is still unclear (for example, in tumors having an unclassified variant mutation) or in tumors in which no mutation has yet been found but where a hereditary factor is suspected.

[098] The BRCA2 classifiers can also be used to diagnose phenotypes relating to BRCA2-associated tumors in HBOC patient families that otherwise test negative for BRCA2-

related mutations using tests and/or screens currently available. As shown in the Examples, when the BRCA2 classifiers were used to test a pool of HBOC diagnosed cases, several presented a positive BRCA2-like profile, indicating that the BRCA2 classifiers were able to detect the involvement of BRCA2, whereas the tests used to make the original diagnoses could not. Additionally, in the same pool of HBOC diagnosed cases tested with the BRCA2 classifiers, a few cases displayed indications for BRCA2-deficiency, indicating that BRCA2 might be involved in these tumors. The BRCA2 classifiers are thus more sensitive and capable of detecting a BRCA2-like profile in tumors than current tests and/or diagnostics. The BRCA2 profiles can be used in addition to known tests and/or diagnostics, to improve results, or in lieu of such tests and diagnostics as an accurate test for BRCA2-related tumors in and of themselves.

[099] Additionally, the BRCA2 classifiers can be used to identify and diagnose sporadic tumors having a BRCA2 profile, as the BRCA2 profile is, in fact, a phenotype of BRCA2 dysfunction. As shown in the Examples, when used in a clinical setting, the BRCA2 classifiers can be used to detect the presence of a BRCA2 profile in triple negative, basal-like sporadic tumors. Additionally, the BRCA2 classifiers can be used to detect the presence of a BRCA2 profile in estrogen receptor positive luminal sporadic tumors.

[0100] In further aspects, the present disclosure relates to kits for use in the diagnostic applications described above. The kits can comprise any or all of the reagents to perform the methods described herein. The kits can comprise one or more of the BRCA2 classifiers, which in some embodiments are present in one or more arrays, as described herein. In the diagnostic applications such kits may include any or all of the following: assay reagents, buffers, nucleic acids such as hybridization probes and/or primers that specifically bind to at least one of the genomic locations described herein, as well as arrays comprising such nucleic acids. In addition, the kits may include instructional materials containing directions (i.e., protocols) for the practice of the methods of this disclosure. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this disclosure. Such media include, but are not limited to electronic storage media (e.g., magnetic discs, tapes, cartridges, chips), optical media (e.g., CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

Examples

[0101] The following examples describe in detail certain embodiments of the BRCA2 arrays and the BRCA2 aCGH classifiers. It will be apparent to those skilled in the art that many modifications, both to materials and methods, may be practiced without departing from the scope of the disclosure.

Example 1

Prediction of BRCA2 association in hereditary breast carcinomas with array-CGH

[0102] A BRCA2 classifier (**Fig. 2**) was built using array-CGH profiles of 28 BRCA2 mutated and 28 sporadic breast tumors. This classifier was validated on an independent group consisting of 19 BRCA2-mutated and 19 sporadic breast tumors. Subsequently, 89 breast tumors from suspected hereditary breast (and ovarian) cancer (HBOC) families in which either no BRCA1/2 mutation or an unclassified variant (UV) had been found by standard diagnostics were tested with this classifier.

[0103] The classifier showed a sensitivity of about 89% and specificity of about 84%. Of the 89 HBOC cases, 17 presented a BRCA2-like profile. In three of these cases, additional indications for BRCA2 deficiency were found. Chromosomal aberrations that were specific for BRCA2-mutated tumors included loss on chromosome arm 13q and 14q, and gain on 17q.

[0104] Use of the classifier to classify breast tumors can be applied as a clinical test, for example for use in addition to current diagnostics, to help clinicians in decision making related to treatment options and in classifying sequence variants of unknown significance.

[0105] Individuals that inherit a germline mutation in BRCA2 will have an increased lifetime risk of developing breast or ovarian cancer. Several recent publications have reviewed the importance of identifying BRCA2 mutation carriers for optimal therapy and non-carriers for chemoprevention (Foulkes, WD. BRCA1 and BRCA2: chemosensitivity, treatment outcomes and prognosis. Fam Cancer 2006;5(2):13542; and Rubinstein WS. Hereditary breast cancer: pathobiology, clinical translation, and potential for targeted cancer therapeutics. Fam Cancer 2008;7(1):839.). Successful mutation identification impacts not only on the patient but also on the family members, since it allows for presymptomatic mutation screening. The current strategy to identify mutation carriers is first to select those patients eligible for mutation screening based on prediction models that use age and family history (Antoniou AC, Hardy R, Walker L, Evans DG, Shenton A, Eeles R, et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet 2008 Jul;45(7):42531). Subsequently, the mutation screening is performed by, for example, sequencing of gene fragments in germline DNA, Protein

Truncation Test (PTT) and Denaturing Gradient Gel Electrophoresis (DGGE) (Hogervorst FB, Cornelis RS, Bout M, van VM, Oosterwijk JC, Olmer R, et al. Rapid detection of BRCA1 mutations by the protein truncation test. Nat Genet 1995 Jun;10(2):20812; and van der Hout AH, van den Ouweland AM, van der Luijt RB, Gille HJ, Bodmer D, Bruggenwirth H, et al. A DGGE system for comprehensive mutation screening of BRCA1 and BRCA2: application in a Dutch cancer clinic setting. Hum Mutat 2006 Jul;27(7):65466.). However, it still remains unclear to what extent mutation carriers are accurately identified with the current diagnostic tools, since many families with a history for breast cancer remain unexplained. It is known that mutation prediction models do not perform perfectly and are highly dependent on the number of family members, from which information is available (Antoniou AC, Hardy R, Walker L, Evans DG, Shenton A, Eeles R, et al. Predicting the likelihood of carrying a BRCA1 or BRCA2 mutation: validation of BOADICEA, BRCAPRO, IBIS, Myriad and the Manchester scoring system using data from UK genetics clinics. J Med Genet 2008 Jul;45(7):42531; and Kang HH, Williams R, Leary J, Ringland C, Kirk J, Ward R. Evaluation of models to predict BRCA germline mutations. Br J Cancer 2006 Oct 9;95(7):91420). Another clinically difficult situation is the identification of a UV in coding or non-coding regions in the BRCA2 gene. The pathogenicity of such a nucleotide variant is often uncertain as the effect on the protein function is unknown. Therefore, its clinical significance also remains unclear. Although functional assays exist for the proteins produced by mutated BRCA2 genes, these are laborious, difficult to interpret in clinical terms, limited to only a number of protein functionalities, and not yet routinely applicable in a diagnostic setting. Therefore, the profiling of somatic genetic changes in breast tumors as described herein provides a new strategy that can give additional information about the involvement of BRCA2 in tumorigenesis.

[0106] In this Example, array-CGH was used to investigate the copy number changes of DNA sequences extracted from formalin fixed, paraffin embedded (FFPE) tissue, which is readily available in pathology archives and therefore very suitable for diagnostic purposes.

[0107] **Materials and Methods**

[0108] **Patient selection**

[0109] Three breast cancer groups were used: 1) 47 breast carcinomas from women with a confirmed pathogenic BRCA2 germline mutation, mean age at diagnosis of 46 years (age range: 26-86); 2) 47 sporadic breast tumors from women with unknown BRCA2 status, mean age at diagnosis of 45 years (age range: 29-78), no known family history for breast cancer and matched to the tumor group mentioned above; 3) 89 tumors from women that

were eligible according to the HBOC criteria for, and subjected to, routine diagnostic testing but were found to be negative for pathogenic BRCA2 mutations, or were diagnosed to carry an UV in BRCA2 (see Table 1), mean age at diagnosis of 47 years (age range: 27-75).

Table 1 – Unclassified variants classified with the aCGH classifiers.

Case	Gene	UV	Type	Effect	Classification
PFT2946 (2x)	BRCA2	c.6842-20T>A	Intronic variant	Different splice prediction programs: no effect	Sporadic-like
PFT5737	BRCA2	c.9502-12T>G	Intronic variant	Loss of splice acceptor site, deletion of exon 26	BRCA2-like
PFT6270	BRCA2	c.1395A>C	Silent coding variant	Very likely no effect	Sporadic-like

Listed are the Type and the Effect of the UVs. aCGH profiles were classified with the BRCA2 classifier shown in Fig. 2 (Classification). Case PFT2946 was diagnosed with two primary tumors.

[0110] All sample material was formalin fixed, paraffin embedded (FFPE) archival tissue; DNA was extracted and the quality tested as described before (Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, et al. Prediction of BRCA1association in hereditary nonBRCA1/2 breast carcinomas with arrayCGH. Breast Cancer Res Treat 2008 Aug 14; and van Beers EH, Joosse SA, Ligtenberg MJ, Fles R, Hogervorst FB, Verhoef S, et al. A multiplex PCR predictor for aCGH success of FFPE samples. Br J Cancer 2006 Jan 30;94(2):3337). The immunohistological characteristics of each tumor group are listed in Table 2, individual sample characteristics are reported in Joosse *et al.*, Prediction of BRCA-2 association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180. All experiments involving human tissues were conducted with the permission of the institute's medical ethical advisory board. CGH profiles of all BRCA1 mutated tumors described in this Example as well as 37 cases of the HBOC group were from a previous study (Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, et al. Prediction of BRCA1association in hereditary nonBRCA1/2 breast carcinomas with arrayCGH. Breast Cancer Res Treat 2008 Aug 14).

Table 2 – Tumor group characteristics. Immunohistological characteristics of the BRCA2-mutated and sporadic tumor groups, listed in percentages. Here intermediate stainings were called positive.

	BRCA2-mutated ($n = 47$)	Sporadic ($n = 47$)	Training B2 ($n = 28$)	Training Sp ($n = 28$)
Grade				
I	15 ($n = 7$)	15 ($n = 7$)	18 ($n = 5$)	14 ($n = 4$)
II	36 ($n = 17$)	32 ($n = 15$)	29 ($n = 8$)	29 ($n = 8$)
III	49 ($n = 23$)	53 ($n = 25$)	54 ($n = 15$)	57 ($n = 16$)
ER				
+	83 ($n = 39$)	83 ($n = 39$)	82 ($n = 23$)	79 ($n = 22$)
-	17 ($n = 8$)	17 ($n = 8$)	18 ($n = 5$)	21 ($n = 6$)
PR				
+	45 ($n = 21$)	57 ($n = 27$)	54 ($n = 15$)	57 ($n = 16$)
-	55 ($n = 26$)	43 ($n = 20$)	46 ($n = 13$)	43 ($n = 12$)
HER2				
+	13 ($n = 6$)	19 ($n = 9$)	18 ($n = 5$)	21 ($n = 6$)
-	87 ($n = 41$)	81 ($n = 38$)	82 ($n = 23$)	79 ($n = 22$)
p53				
+	43 ($n = 20$)	36 ($n = 17$)	86 ($n = 24$)	82 ($n = 23$)
-	57 ($n = 27$)	64 ($n = 30$)	14 ($n = 4$)	18 ($n = 5$)

Values are expressed as percentage.

Training B2 = Classifier training group BRCA2-mutated, Training Sp = Classifier training group Sporadic.

[0111] **Immunohistochemistry (IHC)**

[0112] Presence of ER, PR, HER2/neu, and p53 were determined by immunohistochemistry staining using the following antibodies: estrogen receptor AB14 clone 1D5 + 6F11, titer 1:50 (Neomarkers); progesterone receptor clone PR1, titer 1:400 (Immmunologic); cerbB2 clone SP3, titer 1:25 (Neomarkers); and TP53 clone D07, titer 1:8000 (DAKO), respectively. If ≥70% of the tumor cells expressed ER, PR, or p53, the tumor was scored as positive (+) for the corresponding staining. If ≤10% of the cells were stained, the tumor was scored as negative (-). Those cases that stained between 10% and 70% of the tumor cells were scored as intermediate (+/-) for the corresponding staining. HER2/neu staining was scored positive when a 3+ staining was observed, otherwise it was scored as negative.

[0113] **Array-CGH**

[0114] ULS-Cy5 labeled tumor DNA and ULS-Cy3 labeled female reference DNA were cohybridized for 72 hours on a microarray containing 3.5k BAC/PAC derived DNA segments covering the whole genome with an average spacing of 1MB. Sample preparation, labeling, BAC arrays preparation, and array processing were done as previously described

(Joosse SA, van Beers EH, Nederlof PM. Automated arrayCGH optimized for archival formalinfixed, paraffinembedded tumor material. BMC Cancer 2007;7:43). Microarray data have been deposited in NCBIs Gene Expression Omnibus and are accessible through GEO Series accession number GPL4560.

[0115] **Detection and quantification of aberrations**

[0116] To analyze the chromosomal aberrations, the breakpoint locations and estimated copy number level were determined using the CGH segmentation algorithm described by Picard et al. (Picard F, Robin S, Lavielle M, Vaisse C, Daudin JJ. A statistical approach for array CGH data analysis. BMC Bioinformatics 2005;6:27), further referred to as the 'segmentation data'. Since tumor percentage and heterogeneity both influence the dynamic range of an aCGH profile, a profile dependent cutoff was used for each experiment to call gains and losses instead of an arbitrary chosen cutoff on all samples. The cutoff for every single profile was two times the standard deviation of the profile segmentation data excluding singletons and high level amplification (log2ratio > 1.0) that would otherwise influence the standard deviation excessively. The average of the thresholds was 0.11 (total range: 0.06-0.18). These cutoffs were applied to the segmentation data to calculate the number of aberrations present in a CGH profile. The association of the frequency of a clone being 'gained', 'lost', or 'unchanged' across the different tumors groups was calculated by employing a 3x2 Fisher's exact (FE) test.

[0117] **Classifier**

[0118] The classifier used in this Example is shown in **Fig. 2**. The approach of Dobbin and Simon (Dobbin KK, Zhao Y, Simon RM. How Large a Training Set is Needed to Develop a Classifier for Microarray Data? Clin Cancer Res 2008 Jan 1;14(1):10814) was used to calculate the required sample size using a standardized fold change of 1.3. For an error tolerance of <0.10, more than 23 samples of each class were needed. The Shrunken Centroids (SC) algorithm (Tibshirani R, Hastie T, Narasimhan B, Chu G. Diagnosis of multiple cancer types by shrunken centroids of gene expression. Proc Natl Acad Sci U S A 2002 May 14;99(10):656772) was used to construct the classifier used for array-CGH based on the segmentation data, to eliminate technical noise. To train the classifier, a fraction of 0.6 (n=28) of each group was randomly selected. The classifier was validated with the remaining fraction of the samples (n=19) of each group.

[0119] As a result, the classification algorithm predicts the classes' likelihoods for each sample. Since the sum of the two likelihoods is always "1", the highest class probability (>0.5) is described.

[0120] **Additional screening for BRCA2 defects**

[0121] To identify defects in the BRCA2 gene that could have been missed by standard diagnostics, the following additional tests were performed: BRCA2 exon deletion/duplication MLPA, according to the manufacturer's protocol (MRCHolland, The Netherlands, MLPA kit P090); sequencing of mRNA extracted from lymphocytes to determine bi/monoallelic expression of BRCA2 in the patient along regions containing a single nucleotide polymorphism (SNP), using standard protocols; loss of heterozygosity (LOH) of the BRCA2 locus in tumor DNA using the markers D13S171, D13S260, D13S267, and D13S289; and methylation of the BRCA2 promoter using methylation MLPA according to the manufacturer's protocol (MRCHolland, The Netherlands, MSMLPA kit ME001B).

[0122] **Results**

[0123] Array CGH profiles of 47 BRCA2 mutated, 47 sporadic, and 89 nonBRCA1/2 mutated breast tumors from patients from hereditary breast (and ovarian) cancer families (HBOC) were obtained. The chromosomal aberrations and their locations, the differences between tumor groups, and the discriminating power of a class predictor based on array CGH results are described below.

[0124] **Chromosomal aberrations: BRCA2 vs. sporadic**

[0125] Most aberrations found in the BRCA2-mutated tumor group were also present in the sporadic tumor group in similar frequencies. Genome wide frequency of gains and losses in the BRCA2-mutated and the sporadic control groups were as previously shown (*see* Figure 1, Joosse *et al.*, Prediction of BRCA-2 association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). Based on such frequencies, Fisher's exact test was employed to determine which aberrations are significantly different between the two groups. By this, 7 chromosomal regions were identified as BRCA2-related. These regions consisted of at least 5 adjacent BAC clones with a p-value of <0.01 (Table 3). Based on the calculated breakpoints using CGH-segmentation (Picard F, Robin S, Lavielle M, Vaisse C, Daudin JJ. A statistical approach for array CGH data analysis. BMC Bioinformatics 2005;6:27), the number of aberrations in both tumor groups were counted. BRCA2-mutated tumors showed on average 28.7 aberrations (range: 14-51) and sporadic tumors showed a comparable average of 27.7 aberrations (range: 13-45), which was not significantly different ($p=0.51$, t-test).

Table 3 – Significant chromosomal aberrations. Seven chromosomal regions were present in significantly different frequencies between the mutated and sporadic breast tumors calculated by Fisher's exact (FE) test.

Chr	Cytoband	BRCA2-Mutated		Sporadic		FE test (*p* value)
		Gain (%)	Loss (%)	Gain (%)	Loss (%)	
13	q12-q14	4	78	5	44	2.1E-3
14	q23.2-q32.2	2	62	9	22	5.7E-4
16	p13	14	2	41	3	3.7E-3
16	q12	10	18	5	51	3.0E-3
17	q11-q21.31	36	8	15	32	6.2E-3

Five chromosomal regions (Chr.) were present in significantly different frequencies between the BRCA2-mutated and sporadic breast tumors calculated by Fisher's exact test. Given are the average percentages of gain and loss in both tumor groups of the corresponding chromosomal region and p value (FE test).

[0126] **Chromosomal aberrations: BRCA2 vs. BRCA1**

[0127] Comparison of the CGH profiles of the BRCA2-mutated tumors analyzed in this Example with BRCA1-mutated tumors previously characterized reveals many different aberrations, as shown by the results of Fisher's exact test (*see* Figure 1, Joosse *et al.*, Prediction of BRCA-2 association in hereditary breast carcinomas using array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). The number of aberrations differed significantly between these groups (p=1.25*10-7, t-test), as BRCA2-mutated tumors showed on average 28.7 aberrations, BRCA1-mutated tumors showed on average 36.7 aberrations (range: 22-49).

[0128] **BRCA2 class predictor**

[0129] Twenty-eight CGH profiles of the BRCA2-mutated tumor group and 28 from the sporadic tumor group were randomly selected to train a BRCA2/sporadic breast tumor classifier. Employing leave-one-out cross-validation (LOOCV), Δ =0.4 led to the lowest misclassification rate. Using these 56 profiles, 704 features were selected as discriminatory by the SC algorithm (**Fig. 2**). These features were most abundant along the chromosomal regions 10q23.1-q26.13, 11q13.2-q14.2, 11q23.1-q25, 13q12.2-q21.1, 13q31.3-q33.2, 14q23.2-q32.33, 16p12.1-q21, 17p12-q21.31, 22q11.23-22q13.1, and Xp22.33-p11.3. When reclassifying the training samples, one sample of the BRCA2-mutated tumors and one sample of the sporadic tumors classified to the other class (misclassification of 4%).

[0130] The remaining profiles of 38 samples were used to validate the classifier. **Fig. 2** shows the distribution of the classification scores for the training as well as for the validation sets. During validation, 17 of 19 BRCA2-mutated tumors and 16 of 19 sporadic

tumors were correctly classified. Based on these numbers, the sensitivity was determined to be about 89% and the specificity about 84%; the positive (PPP) and negative predictive power (NPP) were about 85% and about 89%, respectively.

[0131] To further evaluate the performance of the chromosomal regions that were selected for discriminating BRCA2-mutated and sporadic breast tumors, hierarchical cluster analyses (complete linkage, Pearson correlation) was performed on the segmentation data of all the samples based on these regions only. Figure 3 of Joosse *et al.* (Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180) depict the result of the cluster analyses and shows that the samples are divided into three large clusters. IHC data of each sample are displayed along the cluster tree to explore whether samples of both groups residing in one cluster would share the same IHC phenotype, but this was not the case. Two branches contain all except two of the sporadic cases and one large cluster contains all but two of the BRCA2-mutated cases. These results indicate that the features selected for classification do indeed have discriminatory power, regardless of the algorithm and IHC phenotype.

[0132] **Clinical application of the BRCA classifiers**

[0133] To evaluate the BRCA2 classifier in a clinical setting, 89 breast cancer samples from HBOC patients were analyzed. These samples were also classified using a BRCA1 classifier previously described (Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, et al. Prediction of BRCA1association in hereditary nonBRCA1/2 breast carcinomas with arrayCGH. Breast Cancer Res Treat 2008 Aug 14) to investigate the performance of both the classifiers in respect to each other. Using the BRCA2 classifier, seventeen cases (19%) classified as BRCA2-like with a BRCA2 class probability >0.5, 13 of them with a probability >0.8; the remaining 72 cases (81%) were classified as sporadic-like. One of the BRCA2-like cases carried the BRCA2 UV c.950212T>G. When the same 89 cases were tested with the BRCA1 classifier, eleven were diagnosed as BRCA1-like. Of these eleven cases, one carried the BRCA1 UV c.819C>G and two were also classified as BRCA2-like. All 17 BRCA2-like cases, 11 BRCA1-like cases and the cases carrying an UV were studied in more detail using additional molecular tests to identify possible missed BRCA1/2 associated cases, described below.

[0134] **Unclassified variants**

[0135] Sequence analysis had previously revealed unclassified variants in BRCA2 in three of the samples tested (Table 1). To investigate the pathogenicity of these UVs, the mRNA was analyzed by cDNA sequencing. This revealed that BRCA2 UV c.950212T>G led to the deletion of exon 26, indicating that this unclassified variant is pathogenic and results in

nonfunctional proteins. This is in correlation with the CGH profiles of these cases that were classified as BRCA2--like. For the remaining two BRCA2 UV cases, no indications were found for pathogenicity, which is in concordance with the classifier's prediction for the samples, which was sporadic-like.

[0136] **Mutation analysis**

[0137] The entire BRCA2 gene was investigated for whole exon deletions or duplications using the P090 MLPA kit (MRCHolland). None of the investigated cases showed such aberration.

[0138] **Loss of heterozygosity (LOH)**

[0139] LOH was investigated at 4 microsatellite markers flanking the BRCA2 gene in the BRCA2-like cases. Most of the samples (80%) showed LOH at at least one informative (i.e. heterozygous) marker.

[0140] **Promoter methylation**

[0141] Methylation of the BRCA2 promoter was investigated using the ME001 methylation MLPA kit (MRCHolland). None of the HBOC cases were found to be positive for methylation of the BRCA2 promoter.

[0142] **Allele-specific expression**

[0143] It was determined whether both or just one allele of BRCA2 was expressed in the patients' blood. In various embodiments, expression of only one allele could indicate a defective gene by a germline mutation. mRNA regions containing a SNP that was detected by standard diagnostics were sequenced to identify the ratio of expressed alleles. Eight of the BRCA2-like cases were found to be heterozygous for a coding SNP. Only a single case appeared to express one allele of BRCA2, suggesting that this patient carries a defective copy of BRCA2 in her germline DNA.

[0144] **Discussion**

[0145] The chromosomal aberrations of BRCA2-mutated breast tumors were investigated to identify their molecular signature and found that, by using array-CGH, these tumors can be distinguished from sporadic tumors with about 86.5% accuracy. This signature can be used to give additional indications about the involvement of BRCA2 in tumorigenesis of breast tumors where the role of BRCA2 is still unclear (i.e. an UV) or in tumors in which no mutation has yet been found, but where a hereditary factor is suspected. Therefore, via use of the classifier disclosed herein, classification suggesting the involvement of BRCA2 could lead to extended diagnostics, help clinicians in decision making, and lead to adjusted therapy that exploits BRCA2 deficiency.

[0146] Several attempts have been made to identify a molecular BRCA2 signature using gene expression patterns or CGH to discriminate BRCA2-mutated tumors from BRCA1-mutated and sporadic tumors (Jonsson G, Naylor TL, VallonChristersson J, Staaf J, Huang J, Ward MR, et al. Distinct genomic profiles in hereditary breast tumors identified by arraybased comparative genomic hybridization. Cancer Res 2005 Sep 1;65(17):761221; van Beers EH, van WT, Wessels LF, Li Y, Oldenburg RA, Devilee P, et al. Comparative genomic hybridization profiles in human BRCA1 and BRCA2 breast tumors highlight differential sets of genomic aberrations. Cancer Res 2005 Feb 1;65(3):8227; Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, et al. Geneexpression profiles in hereditary breast cancer. N Engl J Med 2001 Feb 22;344(8):53948; and Melchor L, Honrado E, Garcia MJ, Alvarez S, Palacios J, Osorio A, et al. Distinct genomic aberration patterns are found in familial breast cancer associated with different immunohistochemical subtypes. Oncogene 2008 May 15;27(22):316575). BRCA2-mutated tumors are frequently ER positive and grade II, while BRCA1-mutated tumors are in general ER negative and grade III (Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, et al. Prediction of BRCA1association in hereditary nonBRCA1/2 breast carcinomas with arrayCGH. Breast Cancer Res Treat 2008 Aug 14; and Lakhani SR, van de Vijver MJ, Jacquemier J, Anderson TJ, Osin PP, McGuffog L, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002 May 1;20(9):23108). Large parts of the molecular signatures that have been found to discriminate between BRCA1- and BRCA2-mutated breast tumors in previous published studies are also found in sporadic breast tumors that are compared based on ER status or histological grade (Bergamaschi A, Kim YH, Wang P, Sorlie T, HernandezBoussard T, Lonning PE, et al. Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and geneexpression subtypes of breast cancer. Genes Chromosomes Cancer 2006 Nov;45(11):103340; and Melchor L, Honrado E, Huang J, Alvarez S, Naylor TL, Garcia MJ, et al. Estrogen receptor status could modulate the genomic pattern in familial and sporadic breast cancer. Clin Cancer Res 2007 Dec 15;13(24):730513). Comparison of the aCGH profiles of the BRCA2-mutated tumors described herein with the profiles of BRCA1-mutated tumors in previous reports shows many differences of which also many can be related to ER status and histological grade.

[0147] Although BRCA2 is specifically involved in homologous recombination, both the BRCA2-associated and the sporadic tumor group showed a comparable average number

of aberrations (29 and 28 respectively). Several differences between the groups were found based on the frequency of aberrations. These results indicate that loss of function of BRCA2 is not related to more genomic aberrations (detectable with array-CGH) but does require specific genomic locations to be gained or lost in tumorigenesis. Loss on chromosome 14q and the absence of loss on chromosome 16q were found to be significantly different between BRCA2-mutated and sporadic tumors.

[0148] Using the shrunken centroids algorithm, a classifier with BRCA2-mutated and sporadic tumors was built resulting in about 89% sensitivity and about 84% specificity. In order to evaluate the selected centroids, Pearson correlation was used as a second method to investigate the relationship between the samples. This resulted in a total of 4 out of 94 misclassifications, which is comparable with the results obtained with the shrunken centroids algorithm. This indicates that the genomic regions that were selected for the classification are indeed BRCA2 specific, regardless of the algorithm used. Small subclusters within the sporadic class can be distinguished showing separation based on IHC status. It is notable that are the misclassified BRCA2-mutated samples cluster together with sporadic samples sharing similar ER status, again indicating the association of genomic aberrations with ER status.

[0149] Applying the BRCA2 classifier to HBOC cases formally designated as not having either a BRCA1 or a BRCA2 mutation, and to BRCA1 and/or BRCA2 UV carriers, 17 tumors were found to be BRCA2-like. By analyzing germline and tumor DNA from the BRCA2-like cases, as well as mRNA extracted from lymphocytes, indications for dysfunctional BRCA2 were found in three cases. The first of these cases was found to carry an UV that led to the deletion of exon 26 of BRCA2. The other two cases only expressed one BRCA2 allele investigated in lymphocytes. This suggests the presence of a germline defect in the other allele. Methylation of the BRCA2 promoter was not found, however this is in agreement with reports suggesting that this does not occur frequently in breast cancer (Kontorovich T, Cohen Y, Nir U, Friedman E. Promoter methylation patterns of ATM, ATR, BRCA1, BRCA2 and P53 as putative cancer risk modifiers in Jewish BRCA1/BRCA2 mutation carriers. Breast Cancer Res Treat 2008 Jul 19; and Dworkin AM, Spearman AD, Tseng SY, Sweet K, Toland AE. Methylation not a frequent "second hit" in tumors with germline BRCA mutations. Fam Cancer 2009 Apr 2).

[0150] **Conclusion**

[0151] The classifier disclosed herein, as well as the classification method used in this Example were able to distinguish BRCA2-mutated from sporadic breast tumors based on their chromosomal aberrations with an accuracy of about 86.5%. Applying this classifier to

89 breast tumors from high risk patients either carrying no pathogenic BRCA1 and/or BRCA2 mutation or carrying a BRCA2 UV, 17 BRCA2-like cases were identified, from which indicia of BRCA2 deficiency was found in three cases. The classifier can be used as a tool to identify BRCA2-associated patients. The classifier and related methods can be combined with other existing methods in order identify BRCA2-associated patients.

Example 2

Homologous recombination deficiency in breast cancer and association with response to neo-adjuvant chemotherapy

[0152] Tumors with homologous recombination deficiency (HRD), such as BRCA2 associated breast cancers, are not able to reliably repair DNA double strand breaks (DSBs), and are highly sensitive to alkylating agents and PARP inhibitors. Markers that may indicate the presence of HRD in patients with HER2-negative breast cancer, scheduled to receive neoadjuvant chemotherapy, have been previously studied. Forty-three triple negative (TN) and 91 estrogen receptor positive (ER+) pre-treatment biopsies from sporadic breast cancer patients were examined. In ER+ tumors, an aCGH "BRCA2-like" pattern and the amplification of the BRCA2 inhibiting gene EMSY were frequently observed (37% and 15% respectively). In addition, EMSY amplification and a "BRCA2-like" pattern rarely occurred together, raising doubts about the assumption that EMSY amplification inactivates BRCA2 and causes HRD.

[0153] **Introduction**

[0154] The breast cancer gene BRCA2 is involved in homologous recombination and tumors of patients carrying germ-line mutations in this gene show HRD. BRCA2 can be inactivated in sporadic cancers as well (Joosse,S.A., van Beers,E.H., Tielen,I.H., et al Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH, Breast Cancer Res Treat, 2008; and Turner,N., Tutt,A. and Ashworth,A. Hallmarks of 'BRCAness' in sporadic cancers, Nat Rev Cancer, 4: 814-819, 2004), a phenomenon sometimes referred to as "BRCA-ness". Many other genes are involved in homologous recombination, including the Fanconi anemia genes and the BRCA2 inactivating gene EMSY (Hughes-Davies,L., Huntsman,D., Ruas,M., et al EMSY links the BRCA2 pathway to sporadic breast and ovarian cancer, Cell, 115: 523-535, 2003).

[0155] It has previously been shown that breast cancers from BRCA1 mutation carrying patients have a characteristic pattern of DNA gains and losses in an array comparative genomic hybridization (aCGH) assay (Wessels,L.F., van Welsem,T., Hart,A.A., Van't Veer,L.J., Reinders,M.J. and Nederlof,P.M. Molecular classification of breast carcinomas by comparative genomic hybridization: a specific somatic genetic profile for

BRCA1 tumors, Cancer Res, 62: 7110-7117, 2002). This pattern is also found in a subgroup of hormone receptor-negative sporadic breast cancers that do not contain a BRCA1 mutation.

[0156] In this Example, the frequency in which these possibly HRD-associated features occur in untreated patients with breast cancer was prospectively determined.

[0157] **Patients and methods**

[0158] **Patients**

[0159] Pre-treatment biopsies of primary breast tumors from 134 women with HER2 negative breast cancer were collected. All patients had received neoadjuvant treatment at the Netherlands Cancer Institute between 2000 and 2007 as part of two ongoing clinical trials, or were treated off protocol according to the standard arm of one of these studies. Both studies had been approved by the ethical committee and written informed consent was obtained. For eligibility, breast carcinoma with either a primary tumor size of at least 3 cm was required, or the presence of fine needle aspiration (FNA) -proven axillary lymph node metastases. Biopsies were taken using a 14G core needle under ultrasound guidance. After collection, specimens were snap-frozen in liquid nitrogen and stored at -70°C. Each patient had two or three biopsies taken to assure that enough tumor material was available for both diagnosis and further study.

[0160] Depending on the particular study, a treatment regimen was assigned to each patient, which consisted of one of the following: 1.) Six courses of dose-dense Doxorubicin/Cyclophosphamide (ddAC); or 2.) Six courses of Capecitabine/Docetaxel (CD); or 3.) Three courses of ddAC followed by three courses CD (or vice versa) if the therapy response was considered unfavorable by MRI evaluation after three courses. For the response analysis, only those patients who started with ddAC (group 1 and group 3) were considered.

[0161] **Response evaluation**

[0162] The response of the primary tumor to chemotherapy was evaluated by contrast-enhanced MRI after 3 courses of chemotherapy, and after surgery by pathologic evaluation of the resection specimen. The primary end point of both studies was termed a "pCR," which was defined as the complete absence of residual invasive tumor cells seen at microscopy. If only non-invasive tumor (carcinoma in situ) was detected, this was considered a pCR as well. When a small number of scattered tumor cells were seen, the samples were classified as 'near pCR' (npCR). Because the aim of this study was to determine if HRD was correlated with a higher sensitivity to chemotherapy, tumors with a npCR were included in the group of complete remission for analytical purposes. Patients with larger amounts of residual tumor left were classified as non-responders (NR).

[0163] **Array-CGH**

[0164] Tumor DNA and reference DNA were co-hybridized using two different CyDyes to a microarray containing 3.5k BAC/PAC derived DNA segments covering the whole genome with an average spacing of 1MB and processed as described before (Joosse,S.A., van Beers,E.H. and Nederlof,P.M. Automated array-CGH optimized for archival formalin-fixed, paraffin-embedded tumor material, BMC Cancer, 7: 43, 2007). Classification of subtypes was performed using the aCGH BRCA2 classifiers disclosed herein and developed by Joosse *et al.* (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA1-and BRCA2-association in hereditary breast carcinomas with array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). When the BRCA2 score was 0.50 or higher the tumour was qualified as BRCA2-like (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA1-and BRCA2-association in hereditary breast carcinomas with array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). Under this cut-off a tumour was called sporadic-like. For response analysis, a 0.8 cut-off was also applied.

[0165] **RT PCR**

[0166] mRNA isolation and extraction were performed using RNA Bee, according to the manufacturers protocol (Isotex, Friendswood, TX). A 5 μm section halfway through the biopsy was stained for Hematoxylin and Eosin and analyzed by a pathologist for tumor cell percentage. Only samples that contained at least 60% tumor cells were included in further analysis. GAPDH and B-actin were measured for normalization purposes and the average of both gene expression values was used.

[0167] **MLPA**

[0168] Amplification of EMSY (C11orf30) was determined using a custom MLPA set, containing seven different EMSY probes and nine reference probes (MRC Holland, The Netherlands; X025). This EMSY MLPA set was first validated by an EMSY FISH assay (Dako, Glostrup, Denmark). From the comparison of the EMSY FISH assay and the MLPA, it was determined that an average of the seven probes above 1.5 corresponded to EMSY amplification, as detected by at least 6 copies of the probe at the FISH assay. DNA fragments were analyzed on a 3730 DNA Analyzer (AB, USA). For normalization and analysis the Coffalizer program was used (MRC-Holland, The Netherlands).

[0169] **Statistical tests**

[0170] The Fisher's exact test was used to assess association between the dichotomized HRD characteristics. The Mann-Whitney U test was used to analyze means of variables. All data analyses were performed using SPSS version 15.

[0171] **Results**

[0172] **Overview of samples**

[0173] In the series of patients described in this Example, the frequency of features associated with HRD in pre-treatment biopsies was examined. HER2+ tumors were not investigated. aCGH was used to assess "BRCA-ness". If the pattern of genomic alterations resembled that of BRCA2-associated tumors, the sample was called BRCA2-like. If no pattern was recognized the tumor was called sporadic-like. A total of 134 tumors were studied, of which 91 were ER+ and 43 were Triple Negative tumors. See table 1 for an overview of the different patients.

Table 1 Patient and tumor characteristics

		TN		ER+	
Number of patients		43		91	
Median age (sd)		45 (11.18)		50.5 (9.14)	
Progesterone receptor	Positive	0	0 %	58	64%
	Negative	100	100 %	33	36%
T-stage	T1	2	5%	12	13%
	T2	29	67%	51	56%
	T3	11	26%	25	28%
	T4	1	2%	3	3%
N-stage	Node negative	28	65%	22	24%
	Node positive	15	35%	69	76%
Initial chemotherapy	AC	38	88%	81	89%
	DC	2	5%	7	8%
	other	3	7%	3	3%
Response	pCR	15	34%	6	7%
	npCR	7	16%	12	13%
	NR	19	44%	67	74%
	unknown	2	5%	6	7%

AC=doxorubicin, cyclophosphamide; DC=docetaxel, capecitabine; (n)pCR=(near) pathological complete remission; NR= non response

[0174] Array CGH was performed in 37 TN and 75 ER+ tumors. The BRCA2-like profile was observed in both TN and ER+ tumors (32% and 37% respectively) (Table 2). The BRCA2 inhibiting gene EMSY was only amplified in ER+ tumors, in this tumor group the

frequency was 15%. This initial analysis shows that a BRCA2-like profile occurs in both TN and ER+ tumors. This is in concordance with the fact that tumors in BRCA2 carriers are often ER+ (Chappuis,P.O., Nethercot,V. and Foulkes,W.D. Clinico-pathological characteristics of B, Semin Surg Oncol, 18: 287-295, 2000).

Table 2 Summary of HRD characteristics

	TN (n=43)	ER+ (n=91)	p-value
aCGH BRCA2-like			
B2-like	12 (32%)	28 (37%)	
Sp-like	25 (66%)	47 (63%)	0.832
EMSY Amplification			
Amplification	0 (0%)	9 (15%)	
No amplification	23 (100%)	51 (85%)	**0.057**

[0175] **ER+ tumors and BRCA2-like profile and EMSY amplification**

[0176] Table 3 gives an overview of HRD characteristics in ER+ tumors. Many ER+ tumors show a BRCA2-like pattern or an amplification of the BRCA2 inactivating protein EMSY. Interestingly, a BRCA2-like pattern and EMSY amplification occur only in one tumor sample together (Table 3).

Table 3. Overview of HRD characteristics in ER+ tumors*

Sample Number	BRCA2 like	EMSY amplification
2055	-	-
2105	-	-
2099	+	+
2013	+	
2016	+	
2017	+	
2032	+	
2044	+	
2114	+	
2138	+	
2147	+	
100	+	-
158	+	-
2062	+	-
2065	+	-
2071	+	-
2073	+	-
2075	+	-
2077	+	-
2085	+	-
2098	+	-
2117	+	-
2122	+	-
2128	+	-
2143	+	-
2144	+	-
2151	+	-
2153	+	-

Sample Number	BRCA2 like	EMSY amplification
2081	+	-
2100	+	-
2086	-	+
2087	-	+
110	-	+
112	-	+
2038	-	+
2058	-	+
2084	-	+
2120	-	+
2023	-	.

*Only samples with at least one characteristic are shown

[0177] Table 4 gives an overview of HRD characteristics related to clinical pathological factors. It was determined whether BRCA2 and EMSY were related to PR positivity, T-stage, and N-stage. For a BRCA2 pattern, no association was observed for PR positivity, T-stage and N-stage.

Table 4 Association between BRCA2 pattern and EMSY amplification and clinical pathological variables in ER+ tumor samples.

	BRCA2 like pattern			EMSY		
	BRCA2-like	Sporadic-like	p-value	Amplification	No amplification	p-value
PRpos	15/27 (56%)	36/47 (77%)	0.072	7/9 (78)	34/51 (68)	0.71
T-stage						
1	2/28 (7%)	8/48 (17%)		0 (0%)	8/51 (16%)	
2	18/28 (64%)	26/48 (54%)		5/9 (56%)	28/51(55%)	
3	7/28 (25%)	13/48 (27%)		4/9 (44%)	14/51 (28%)	
4	1/28 (4%)	1/48 (2%)		0	1/51 (2%)	
N-stage						
Pos	19/28 (68%)	41/48 (83%)	0.086	7/9 (78%)	41/51 (80%)	1

[0178] **Discussion**

[0179] Classical chemotherapeutic agents that cause DNA double-strand breaks (DSBs) are thought to be particularly effective in tumors with HRD (Kennedy,R.D., Quinn,J.E., Mullan,P.B., Johnston,P.G. and Harkin,D.P. The role of BRCA1 in the cellular response to chemotherapy, J Natl Cancer Inst, 96: 1659-1668, 2004; Fedier,A., Steiner,R.A., Schwarz,V.A., Lenherr,L., Haller,U. and Fink,D. The effect of loss of Brca1 on the sensitivity to anticancer agents in p53-deficient cells, Int J Oncol, 22: 1169-1173, 2003; Helleday,T., Petermann,E., Lundin,C., Hodgson,B. and Sharma,R.A. DNA repair pathways as targets for cancer therapy, Nat Rev Cancer, 8: 193-204, 2008; Moynahan,M.E., Cui,T.Y. and Jasin,M. Homology-directed dna repair, mitomycin-c resistance, and chromosome stability is restored with correction of a Brca1 mutation, Cancer Res, 61: 4842-4850, 2001; and Powell,S.N. and Kachnic,L.A. Therapeutic exploitation of tumor cell defects in

homologous recombination, Anticancer Agents Med Chem, 8: 448-460, 2008) and the novel class of PARP inhibiting drugs has been shown to have marked antitumor activity with very little toxicity (Bryant,H.E., Schultz,N., Thomas,H.D., et al Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase, Nature, 434: 913-917, 2005; and Farmer,H., McCabe,N., Lord,C.J., et al Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy, Nature, 434: 917-921, 2005). Unfortunately, a demonstration of HRD in clinical tumor samples is problematic. One reported assay measures DSB repair pathways, but requires short-term cultures of primary breast cancer cells (Keimling,M., Kaur,J., Bagadi,S.A., Kreienberg,R., Wiesmuller,L. and Ralhan,R. A sensitive test for the detection of specific DSB repair defects in primary cells from breast cancer specimens, Int J Cancer, 123: 730-736, 2008). Immunohistochemical methods have been proposed as well, aiming to detect CHK1 and RAD51 localization in the cytoplasm and/or the nucleus (Honrado,E., Osorio,A., Palacios,J., et al Immunohistochemical expression of DNA repair proteins in familial breast cancer differentiate BRCA2-associated tumors, J Clin Oncol, 23: 7503-7511, 2005), but reliable immunohistochemical staining results can be difficult to obtain. Others have used methylation assays for BRCA1 (Esteller,M., Silva,J.M., Dominguez,G., et al Promoter hypermethylation and BRCA1 inactivation in sporadic breast and ovarian tumors, J Natl Cancer Inst, 92: 564-569, 2000; and Catteau,A., Harris,W.H., Xu,C.F. and Solomon,E. Methylation of the BRCA1 promoter region in sporadic breast and ovarian cancer: correlation with disease characteristics, Oncogene, 18: 1957-1965, 1999), FancC and FancD and have studied EMSY amplification (Rodriguez,C., Hughes-Davies,L., Valles,H., et al Amplification of the BRCA2 pathway gene EMSY in sporadic breast cancer is related to negative outcome, Clin Cancer Res, 10: 5785-5791, 2004), e.g. by an in situ hybridization assay (Turner,N., Tutt,A. and Ashworth,A. Hallmarks of 'BRCAness' in sporadic cancers, Nat Rev Cancer, 4: 814-819, 2004). The sensitivity and specificity of these approaches is unknown and a possible association of these features with neoadjuvant treatment response has not been reported.

[0180] High-dose alkylating chemotherapy in the treatment of patients with breast cancer, with either a high risk of relapse (Rodenhuis,S., Bontenbal,M., Beex,L.V., et al High-dose chemotherapy with hematopoietic stem-cell rescue for high-risk breast cancer, N Engl J Med, 349: 7-16, 2003) or with distant metastases (Schrama,J.G., Baars,J.W., Holtkamp,M.J., Schornagel,J.H., Beijnen,J.H. and Rodenhuis,S. Phase II study of a multi-course high-dose chemotherapy regimen incorporating cyclophosphamide, thiotepa, and carboplatin in stage IV breast cancer, Bone Marrow Transplant, 28: 173-180, 2001), has been previously

reported. In both studies, a modest survival advantage for patients who had received this intensive treatment was observed, a result which has also been documented in meta-analyses of the randomized studies (Berry,D.A., Ueno,N.T., Johnson,M.M., et al High-dose chemotherapy with autologous stem-cell support versus standard-dose chemotherapy: meta-analysis of individual patient data from 6 randomized metastatic breast cancer trials, Proc.San Antonio Breast Cancer Symp, Abstract 6113:2008). These observations are consistent with the existence of a putative subgroup of breast cancers that is highly responsive to alkylating drugs (Rodenhuis,S. The status of high-dose chemotherapy in breast cancer, Oncologist, 5: 369-375, 2000; and Rodenhuis,S. High-dose chemotherapy in breast cancer--interpretation of the randomized trials, Anticancer Drugs, 12: 85-88, 2001).

[0181] In the series of patients described in this Example, the frequency of certain features associated with HRD in untreated breast cancers was studied. BRCA2 inactivation, shown by a BRCA2 like aCGH profile and EMSY amplification, was specifically observed in ER+ tumors.

[0182] **Features of BRCA2 inactivation**

[0183] Of the ER+ and TN tumors combined, roughly one-third had a BRCA2-like profile, while EMSY amplification was exclusively found in the ER+ tumors. In a series of 183 breast tumors from BRCA2 mutation carriers and from sporadic breast tumors, BRCA2 methylation has been assessed, but methylation was not found in any of the samples (Joosse,S.A., Brandwijk,K.I.M., Devilee,P., et al Prediction of BRCA1-and BRCA2-association in hereditary breast carcinomas with array-CGH, Breast Cancer Res Treat. 2010 Jul 8. PubMed PMID: 20614180). In the literature, BRCA2 promotor methylation has been sporadically observed in ovarian cancer (Hilton,J.L., Geisler,J.P., Rathe,J.A., Hattermann-Zogg,M.A., DeYoung,B. and Buller,R.E. Inactivation of BRCA1 and BRCA2 in ovarian cancer, J Natl Cancer Inst, 94: 1396-1406, 2002), but not in breast cancer. An alternative mechanism for BRCA2 inactivation involves amplification of the EMSY gene. Interestingly, the present study did not identify overlap between tumors showing a BRCA2-like profile and EMSY amplification, except for one case (Table 3). This observation points at two different routes or levels of BRCA2 inactivation. In tumors with EMSY amplification, usually a lower degree of chromosomal gains and losses is observed than in the BRCA2-like tumors. Moreover, in a different series of 52 sporadic tumors from which aCGH data are available at the Netherlands Cancer Institute, 7 ER+ tumors with a gain at the EMSY locus were detected, and none of these showed a BRCA2 like profile. This supports the finding that EMSY and the BRCA2 like profile only rarely occur together and that EMSY amplification is not

associated with the same degree of chromosomal instability as BRCA2 mutation. In vitro assays have shown that the EMSY protein can bind BRCA2 protein and inactivate its function (Raouf,A., Brown,L., Vrcelj,N., et al Genomic instability of human mammary epithelial cells overexpressing a truncated form of EMSY, J Natl Cancer Inst, 97: 1302-1306, 2005). An increase in chromosomal instability was observed after EMSY overexpression. However, it is not clear if EMSY amplification affects the role of BRCA2 in the maintenance of genomic instability in vivo, which may depend on the levels of both proteins and their cellular localization.

[0184] **Conclusion**

[0185] In ER+ tumors, an aCGH "BRCA2-like" pattern and the amplification of the BRCA2 inhibiting gene EMSY were frequently observed (37% and 15% respectively). In addition, EMSY amplification and a "BRCA2-like" pattern rarely occurred together, raising doubts about the assumption that EMSY amplification inactivates BRCA2 and causes.

[0186] Finally, it should be noted that there are alternative ways of implementing the embodiments disclosed herein. Accordingly, the present embodiments are to be considered as illustrative and not restrictive. Furthermore, the claims are not to be limited to the details given herein, and are entitled their full scope and equivalents thereof.

Claims

What is claimed is:

1. A method of classifying a tumor, comprising:

obtaining a test sample from a patient;

detecting the copy numbers of DNA in the test sample in at least one of the genomic loci selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28; and

comparing the copy numbers in the test sample to corresponding copy numbers in a reference sample;

wherein a variation in the copy numbers in the test sample in at least one of the genomic loci selected from 6p25.3-11.1, 6q12-13, 10q22.3-26.13, 13q12.2-21.1, 13q31.3-33.1 and 14q23.2-32.33 classifies the test sample as from a BRCA2-associated tumor; and

wherein a variation in the copy numbers in the test sample in at least one of the genomic loci selected from 2q36.3-37.1, 4p13-12, 16p12.3-11.2, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 classifies the test sample as from a sporadic tumor.

2. The method of claim 1, wherein an increase in the copy numbers in the test sample in at least one, or a plurality, of genomic loci selected from 6p25.3-11.1, 6q12-13 and 13q31.3-33.1 classifies the test sample as from a BRCA2-associated tumor.

3. The method of claim 1, wherein a decrease in the copy numbers in the test sample in at least one, or a plurality, of genomic loci selected from 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33 classifies the test sample as from a BRCA2-associated tumor.

4. The method of claim 1, wherein an increase in the copy numbers in the test sample in the genomic locus 16p12.3-11.2 classifies the test sample as from a sporadic tumor.

5. The method of claim 1, wherein a decrease in the copy numbers in the test sample in at least one, or a plurality, of genomic loci selected from 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31 classifies the test sample as from a sporadic tumor.

6. The method of claim 1, wherein the DNA in the test sample is detected in the genomic loci 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2,

11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

7. The method of any one of claims 1-6, wherein the detecting is performed by array comparative genomic hybridization using an array.

8. The method of claim 7, wherein the array comprises a plurality of probes derived from at least one of the BAC clones of **Fig. 2** immobilized on a substrate.

9. The method of claim 8, wherein the probes are derived from at least 100 of the BAC clones of **Fig. 2**.

10. The method of any one of claims 8 and 9, wherein the probes are derived from all 704 of the BAC clones of **Fig. 2**.

11. The method of any one of claims 8-10, wherein the probes independently hybridize to DNA from at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

12. An array comprising a plurality of probes immobilized on a substrate, wherein the probes hybridize to DNA from at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

13. The array of claim 12, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1.

14. The array of claim 13, wherein the probes detect an increase in copy number of the DNA from the genomic loci.

15. The array of claim 12, wherein the probes hybridize to DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33.

16. The array of claim 15, wherein the probes detect a decrease in copy number of the DNA from the genomic loci.

17. The array of claim 12, wherein the probes hybridize to DNA from the genomic locus 16p12.3-11.2.

18. The array of claim 17, wherein the probes detect an increase in copy number of the DNA from the genomic locus.

19. The array of claim 12, wherein the probes hybridize to DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

20. The array of claim 19, wherein the probes detect a decrease in copy number of the DNA from the genomic loci.

21. The array of claim 12, wherein the probes at least hybridize to:

DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33;

DNA from the genomic locus 16p12.3-11.2; and

DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

22. The array of claim 12, wherein the probes hybridize to DNA from the genomic loci 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

23. The array of any one of claims 21 and 22, wherein the probes:

detect an increase in copy number of the DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

detect a decrease in copy number of the DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33;

detect an increase in copy number of the DNA from the genomic locus 16p12.3-11.2; and

detect a decrease in copy number of the DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

24. The array of any one of claims 12-23, wherein the probes are derived from at least 100 of the BAC clones of **Fig. 2**.

25. The array of any one of claims 12-24, wherein the probes are derived from all 704 of the BAC clones of **Fig. 2**.

26. A BRCA2 classifier comprising a plurality of probes, wherein the probes hybridize to DNA from at least one genomic locus selected from 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

27. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1.

28. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33.

29. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic locus 16p12.3-11.2.

30. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

31. The classifier of claim 26, wherein the probes at least hybridize to:

DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33;

DNA from the genomic locus 16p12.3-11.2; and

DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

32. The classifier of claim 26, wherein the probes hybridize to DNA from the genomic loci 2p24.1-16.3, 2q36.3-37.1, 3p12.3-3q11.2, 4p13-12, 6p25.3-11.1, 6q12-13, 7q11.21-11.22, 7q35-36.3, 10p15.2-12.1, 10q22.3-26.13, 11p15.5-15.4, 11q13.2-14.2, 11q23.1-25, 13q12.2-21.1, 13q31.3-33.1, 14q12-21.2, 14q23.2-32.33, 16p12.3-11.2, 16q12.1-21, 17p12-11.2, 17q11.1-12, 17q21.2-21.31, 22q11.23-13.1, 23p22.33-11.3 and 23q26.2-28.

33. The classifier of any one of claims 31 and 32, wherein the probes:

detect an increase in copy number of the DNA from the genomic loci 6p25.3-11.1, 6q12-13 and 13q31.3-33.1;

detect a decrease in copy number of the DNA from the genomic loci 10q22.3-26.13, 13q12.2-21.1 and 14q23.2-32.33;

detect an increase in copy number of the DNA from the genomic locus 16p12.3-11.2; and

detect a decrease in copy number of the DNA from the genomic loci 2q36.3-37.1, 4p13-12, 16q12.1-21, 17q11.1-12 and 17q21.2-21.31.

34. The classifier of any one of claims 26-33, wherein the probes are derived from at least 100 of the BAC clones of **Fig. 2**.

35. The array of any one of claims 26-34, wherein the probes are derived from all 704 of the BAC clones of **Fig. 2**.

FIGURE 1A

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
2	RP11-560C7	RP5-960D23	2p24.1	2p16.3	22998568	47897750	24.9	
2	RP11-86O17	RP11-263G22	2q36.3	2q37.1	226629534	234701766	8.1	loss in SPOR
3	RP11-510B7	RP11-12A13	3p12.3	3q11.2	80611850	96250469	15.6	
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
6	GS-62-L11	RP11-199A24	6p25.3	6p11.1	135996	58788604	58.7	gain in BRCA2
6	RP11-349P19	RP11-256L9	6q12	6q13	65158448	73217550	8.1	gain in BRCA2
7	RP4-756H11	RP4-635O5	7q11.21	7q11.22	65647490	71274716	5.6	
7	RP4-811H12	GS-3-K23	7q35	7q36.3	146787501	158864424	12.1	
10	RP11-118K6	RP13-355A21	10p15.2	10p12.1	3035538	28141734	25.1	
10	RP11-90J7	RP11-436O19	10q22.3	10q26.13	79687950	124268654	44.6	loss in BRCA2
11	RP11-113A6	RP11-327O2	11p15.5	11p15.4	2261568	10454001	8.2	
11	RP11-569N5	RP11-137O10	11q13.2	11q14.2	68072319	87028461	19.0	
11	RP11-108O10	GS-26-N8	11q23.1	11q25	111095144	134437384	23.3	
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-468E2	RP11-168D12	14q12	14q21.2	23572376	41500293	17.9	
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-429K17	RP11-2C24	16p12.3	16p11.2	20151302	30747839	10.6	gain in SPOR
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP1-27J12	RP11-121A13	17p12	17p11.2	14285924	20230382	5.9	
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR
22	RP11-80O7	RP1-172B20	22q11.23	22q13.1	22510132	38559115	16.0	
23	GS-839-D20	RP11-576G22	23p22.33	23p11.3	110000	45268722	45.2	
23	RP5-965E19	RP5-1087L19	23q26.2	23q28	131271371	153547872	22.3	

FIGURE 1B

Chromosome	Begin BAC	END BAC	chrom band	chrom band	region start	Region end	size Mb	characteristic
4	RP11-227F19	RP11-416A5	4p13	4p12	41340065	45778656	4.4	loss in SPOR
13	RP11-125I23	RP11-384G23	13q12.2	13q21.1	26843991	52955532	26.1	loss in BRCA2
13	RP11-95C14	RP11-255P5	13q31.3	13q33.1	91284428	102377477	11.1	gain in BRCA2
14	RP11-544I20	RP11-73M18	14q23.2	14q32.33	63342178	103382895	40.0	loss in BRCA2
16	RP11-283C7	RP11-370P15	16q12.1	16q21	45627546	62396983	16.8	loss in SPOR
17	RP11-260A9	RP11-445F12	17q11.1	17q12	22287134	32371528	10.1	loss in SPOR
17	RP5-1110E20	RP5-971F3	17q21.2	17q21.31	36260511	41939592	5.7	loss in SPOR

FIGURE 2

Clone	Centroids 704 BRCA2	GRCh37 Ensembl56	start	end
RP4-575L21	-0.021125	1	10154043	10246367
RP4-669K10	-0.152518	1	28962129	28962785
RP11-114B7	-0.029022	1	33102443	33273241
RP5-1043G4	0.030203	1	55270329	55270865
RP1-97P20	0.202631	1	169821804	169863408
RP11-100E13	-0.035699	1	224697138	224858861
RP11-528D17	-0.039751	1	221148264	221328294
RP11-399B15	-0.004144	1	245156672	245363768
RP11-438H8	-0.160698	1	247948203	248113393
RP11-560C7	-0.048528	2	23146100	23328443
RP11-557N21	-0.212993	2	23817707	23926090
RP11-557N21	-0.212993	2	23817707	23926090
RP11-169L20	-0.14628	2	24850139	25036031
RP11-404P12	-0.14628	2	25844534	26016317
RP11-106G13	-0.008506	2	26968701	27135673
RP11-373D23	-0.008506	2	28560131	28717580
RP11-328L16	-0.008506	2	29408613	29599239
RP11-559D11	-0.115671	2	32631123	32795421
RP11-299C5	-0.044483	2	42481333	42664230
RP11-421J10	-0.470412	2	45401278	45595215
RP11-27C22	-0.064912	2	46041314	46230526
RP11-110G2	-0.173062	2	46252606	46442514
RP11-436K12	-0.049791	2	47840483	47841282
RP5-960D23	-0.117312	2	47921241	48043172
RP11-30C22	-0.033203	2	54896322	55064464
RP11-304A15	-0.107136	2	69007759	69169681
RP11-304A15	-0.107136	2	69007759	69169681
RP11-542D13	-0.126564	2	98108186	98108876
RP11-547F10	-0.126564	2	98646525	98831782
RP11-549H5	-0.124728	2	100589799	100760360
RP11-30G7	-0.124728	2	100751733	100924841
RP11-298E18	-0.198838	2	124953149	125053149
RP11-48K7	-0.03105	2	127373737	127536405
RP11-535B12	-0.000561	2	180238113	180408717
RP11-507C18	0.001235	2	205007999	205205934
RP11-15J24	0.001235	2	205467646	205638019
RP11-325M10	0.001235	2	206347149	206533169
RP11-86O17	0.294376	2	226921290	227031126
RP11-86O17	0.294376	2	226921290	227031126
RP11-211K17	0.112976	2	227332561	227495985
RP11-149O3	0.017067	2	228659456	228832792
RP11-70L16	0.017067	2	229609402	229766511
RP11-419H23	0.040678	2	232079718	232080385

FIGURE 2

RP11-91N19	0.218862	2	233280034	233434643
RP11-534J17	0.012665	2	234273852	234435410
RP11-534J17	0.012665	2	234273852	234435410
RP11-263G22	0.012665	2	234888946	235035976
RP11-263G22	0.058667	2	234888946	235035976
RP11-97C16	0.035866	3	1373065	1541440
RP11-95E11	0.035866	3	3160172	3328965
RP11-10H6	0.035866	3	3182042	3350580
RP11-183N22	0.035866	3	4517889	4518650
RP11-238A9	0.035866	3	4516514	4676956
RP11-25C10	0.024778	3	12446266	12614814
RP11-163D23	0.024778	3	12616855	12759917
RP11-165B2	0.024778	3	13823746	13988636
RP11-27J5	0.015562	3	20662018	20852567
RP11-170K19	0.002219	3	59727302	59899910
RP11-20B7	0.099057	3	73773215	73942284
RP11-510B7	0.076236	3	80530160	80712228
RP11-442C9	0.023143	3	81727298	81728106
RP11-425D6	0.023143	3	81566843	81567635
RP11-425D6	0.023143	3	81566843	81567635
RP11-206J21	0.023143	3	82574172	82715711
RP11-382L10	0.073911	3	83685332	83685896
RP11-474M18	0.456238	3	84541156	84541982
RP11-447J13	0.37679	3	86006748	86184860
RP11-81P15	0.37679	3	86962850	87140031
RP11-81p15	0.37679	3	86962850	87140031
RP11-312H1	0.228484	3	87488189	87646565
RP11-88I7	0.016342	3	94037937	94222721
RP11-12A13	0.016342	3	94611500	94766779
RP11-58D2	0.057191	3	114797967	114946260
RP11-249J17	0.057191	3	115458843	115626868
RP11-165B13	0.037539	3	117760127	117760888
RP11-572O17	0.347197	4	1626433	1627536
RP11-565I3	-0.12499	4	6945137	6962926
RP11-24H13	0.145506	4	34786915	34955520
RP11-227F19	0.110111	4	41646350	41831334
RP11-109E24	0.255604	4	42636077	42813830
RP11-229G4	0.074035	4	43207054	43307054
RP11-55C6	0.063409	4	44835262	44979919
RP11-416A5	0.004672	4	45914433	46084878
RP11-400D2	0.077091	4	135245617	135420334
RP11-127A9	0.077091	4	136568108	136733118
RP11-63M2	0.013785	4	137796004	137955859
RP11-84H6	-0.046445	4	179170140	179349717
RP11-45F23	-0.056703	4	190284043	190430413
1129-C9	-0.127659	5	50	100050
RP11-57I6	-0.106146	5	23644140	23795207
RP11-57I6	-0.106146	5	23644140	23795207
CTD-2219P12	-0.0508	5	27325194	27427057
RP11-37M16	-0.081588	5	28716023	28881237
CTD-2052F19	-0.160737	5	34894786	35006515

FIGURE 2

CTD-2007F2	-0.035823	5	38165822	38269649
RP11-15A14	-0.035823	5	39191269	39385812
CTD-2276O24	0.199417	5	50026715	50131413
RP4-592P18	0.108382	5	51049080	51195687
RP11-506H20	0.038879	5	54527948	54727328
RP11-210O14	0.071366	5	60167981	60168520
RP11-34J15	-0.074893	5	61624859	61796141
RP11-494C5	0.066886	5	152838083	153003938
RP11-292M11	-0.377792	5	169915012	170086458
84-C11	-0.460991	5	180714187	180814187
GS-62-L11	0.216322	6	135996	235996
GS-196-I5	0.216322	6	250000	350000
RP11-13J16	0.216322	6	1297036	1357295
RP11-15N12	0.216322	6	3348078	3525148
RP11-174B19	0.118521	6	5084698	5271233
RP11-232H4	0.208053	6	6175859	6334512
RP11-163I22	0.208053	6	6273515	6436301
RP11-320C15	0.146965	6	6703139	6859877
RP3-336K20	0.024784	6	7483281	7629676
RP11-196B15	0.208588	6	7954163	8132797
RP3-365E2	0.197931	6	14429170	14564812
RP1-232K4	0.197931	6	15325618	15521703
RP1-13D10	0.375748	6	16058670	16209663
RP11-68J15	0.304161	6	17628621	17771850
RP11-408C8	0.304161	6	19023271	19023868
RP4-625H18	0.218811	6	19726252	19909568
RP3-444C7	0.085716	6	20523819	20650057
RP11-204E9	0.011555	6	21405056	21589578
RP11-33I5	0.202226	6	22473443	22627331
RP1-278E11	0.077917	6	39870200	39969544
RP11-162O6	0.077917	6	40753403	40929365
RP11-533O20	0.180169	6	41890906	42063209
RP11-501I18	0.180169	6	42780788	42846928
RP11-227E22	0.026242	6	43858965	44040979
RP11-554O14	0.030765	6	44880799	45055750
RP3-341E18	0.151441	6	52889011	52979483
RP11-362K18	0.279115	6	53985202	54145854
RP11-524H19	0.279115	6	54613786	54801265
RP11-472M19	0.302324	6	56640449	56817727
RP3-422B11	0.109123	6	57185560	57342705
RP11-199A24	0.016344	6	58503276	58679639
RP11-349P19	0.069561	6	65035120	65097390
RP1-40C9	0.008236	6	65783270	65784054
RP3-324B8	0.225932	6	66821093	66963834
RP11-409K15	0.225932	6	68157449	68297367
RP1-46B1	0.079193	6	69383014	69539129
RP11-462G2	0.169344	6	71009571	71198194
RP11-111D8	0.169344	6	72101590	72284545
RP11-256L9	0.178213	6	73038504	73218079
RP3-443C4	-0.221279	6	152053838	152145741
RP11-450E24	-0.250487	6	152084343	152290880

FIGURE 2

RP1-130E4	-0.250487	6	152478376	152478910
RP4-546K19	-0.110507	6	153064308	153174576
GS-57-H24	0.198508	6	170569992	170669992
RP11-41A3	-0.154226	7	35169796	35253695
RP5-1091E12	-0.207312	7	55042213	55042908
RP4-756H11	0.154232	7	66011128	66144029
RP11-458F8	0.27486	7	66298311	66453955
RP4-736H5	0.383203	7	67030260	67176862
RP11-358M3	0.383203	7	67591342	67591923
RP11-156A14	0.383203	7	67909149	68064977
RP11-3P22	0.383203	7	68614075	68779535
RP11-409J21	0.273299	7	71050338	71203941
RP4-635O5	0.273299	7	71507839	71635768
RP11-101N13	0.027188	7	94478109	94641349
RP5-1145A22	0.0244	7	97264652	97386239
RP11-10E6	0.184172	7	109251622	109404921
RP11-10E6	0.262054	7	109251622	109404921
RP11-391H5	-0.06984	7	127601775	127799120
RP4-811H12	-0.045176	7	147318092	147318704
RP11-302C22	-0.064168	7	147454336	147627247
RP11-24N19	-0.277116	7	148595274	148756871
RP4-800G7	-0.277116	7	148832677	148994476
RP11-511P7	-0.11727	7	150020921	150206768
RP4-548K24	-0.11727	7	150539825	150662642
RP4-548D19	-0.11727	7	150861149	150998990
RP11-312C1	-0.215169	7	152459778	152639200
RP11-312C1	-0.362118	7	152459778	152639200
RP11-317C13	-0.154646	7	153589527	153641465
RP11-269M19	-0.003701	7	154634176	154782516
RP5-1015O24	-0.003701	7	155295097	155490615
RP11-69O3	-0.060486	7	155501974	155654553
RP4-764O12	-0.062889	7	157229624	157342427
RP11-452C13	-0.062889	7	157614278	157824565
GS-3-K23	-0.066477	7	158764424	158864424
GS-580-L5	0.025745	8	200000	300000
GS-77-L23	0.025745	8	300000	400000
RP11-338B22	0.025745	8	488653	667324
RP4-593A12	0.025745	8	1542145	1691889
RP11-297N6	0.326732	8	11578760	11702671
RP11-363L24	0.085846	8	31006261	31006261
RP11-301H15	0.085846	8	32411506	32594737
RP11-11N9	0.008963	8	32908355	32909082
RP11-197P20	-0.30227	8	37086323	37251083
RP11-197P20	-0.30227	8	37086323	37251083
RP11-350N15	-0.653063	8	38170901	38368835
RP11-44K6	-0.143054	8	39718456	39865432
RP11-414L17	-0.00916	8	61514660	61687626
RP11-227F6	-0.130359	8	62249646	62409175
RP11-51M18	0.037368	8	84216776	84386516
RP11-419L20	0.134382	8	110368642	110542334
RP11-28I2	-0.665709	8	127495448	127694132

FIGURE 2

RP11-343P9	0.048876	8	136402189	136583198
RP11-172M18	0.048876	8	139239227	139383045
RP11-526P7	0.057715	8	140217248	140373496
RP11-370K2	-0.067661	8	142544371	142720368
GS-261-I1	-0.118929	8	146054826	146154826
GS-489-D14	-0.118929	8	146054876	146154876
RP11-48M17	0.00548	9	2147364	2305360
RP11-509J21	0.097571	9	3544199	3705631
RP11-125K10	0.220999	9	4830733	5000796
RP11-509D8	0.152012	9	4922574	5130406
RP11-23D5	0.004467	9	11181428	11350967
RP11-123J20	0.282633	9	17850221	18022839
RP11-15P13	0.096009	9	20183465	20360121
RP11-149I2	0.141668	9	21862433	22055818
RP11-264J11	0.328498	9	28850269	28851013
RP11-326F20	-0.029936	9	33086353	33247752
RP11-195F19	-0.029936	9	34634468	34820900
RP11-112J3	-0.029936	9	35670117	35865495
RP11-8N6	-0.029936	9	36632473	36774305
RP11-397D12	-0.029936	9	37263640	37455975
RP11-274B18	0.022824	9	71129855	71297835
RP11-71A24	0.440962	9	75619763	75786792
RP11-439A18	-0.158439	9	86102092	86295020
RP11-59M22	-0.050124	9	86991741	87146621
RP11-172F7	-0.078098	9	88058064	88058760
RP11-440G5	-0.178714	9	94131899	94300747
RP11-23J9	0.11168	9	100042032	100205448
RP11-23B15	0.228212	9	100545008	100703779
RP11-205K6	-0.090263	9	129217477	129380045
GS-135-I17	0.086991	9	140158252	140258252
RP11-118K6	-0.209449	10	3035538	3133935
RP11-154P11	-0.060717	10	4333070	4506152
RP11-336A10	-0.174232	10	5638110	5803775
RP11-298K24	-0.174232	10	6238668	6435633
RP4-542G16	-0.047156	10	7157764	7295862
GS1-756B1	-0.146199	10	8180572	8305520
RP1-249K20	-0.167377	10	9060753	9157064
RP11-189M8	-0.171631	10	9995827	10161417
RP11-566K1	-0.19338	10	10791781	10979028
RP1-251M9	-0.19338	10	10915523	11053430
RP11-401F24	-0.161555	10	11766246	11970786
RP11-730A19	-0.274991	10	13019504	13213671
RP11-275E20	-0.185994	10	14282735	14473313
RP11-2K17	-0.183957	10	14913569	15099778
RP11-37P5	-0.251641	10	15999930	16155788
RP11-337F21	-0.251641	10	16967978	17153153
RP11-16O1	-0.251641	10	17719035	17880741
RP11-383B4	-0.330187	10	18803312	18997209
RP11-188P8	-0.330187	10	19986417	20148469
RP11-337N19	-0.330187	10	20522279	20700003
RP11-165O3	-0.330187	10	21281276	21281882

FIGURE 2

RP11-108B14	-0.330187	10	22360413	22534888
RP11-379L21	-0.40624	10	23084792	23278752
RP11-162E8	-0.376429	10	24120160	24300421
RP11-129O7	-0.323034	10	25101996	25265814
RP11-307B23	-0.100013	10	25930725	26091525
RP11-128B16	-0.003461	10	26758309	26900962
RP13-355A21	-0.096552	10	27896302	28100727
RP11-38B21	-0.008561	10	44545781	44703284
RP11-79I23	-0.083928	10	60426379	60599657
RP11-210G22	0.246152	10	67730039	67909272
RP11-90J7	-0.267551	10	80018953	80173990
RP11-574P20	-0.304665	10	80803824	80971399
RP11-40F6	-0.200148	10	82028900	82029577
RP11-20E23	-0.329272	10	82490215	82657699
RP4-684F13	-0.172354	10	83646484	83647175
RP11-95M17	-0.172354	10	84435533	84586780
RP11-219F10	-0.247379	10	85578672	85756585
RP11-470J18	-0.18939	10	86237532	86399151
RP11-113E21	-0.246808	10	87201090	87382234
RP11-396M20	-0.246808	10	88065908	88231673
RP11-165M8	-0.020621	10	89704489	89704995
RP11-380G5	-0.020621	10	89608026	89807449
RP11-162K11	-0.129274	10	95785188	95933132
RP11-248J23	-0.05225	10	97623987	97789364
RP11-196N24	-0.05225	10	98432174	98534683
RP11-19C6	-0.086945	10	99631313	99790502
RP11-704L16	0.284322	10	100952271	101170323
RP11-287G8	-0.071512	10	101712730	101881251
RP11-324L3	-0.071512	10	102906124	103083779
RP11-165P9	-0.030806	10	106759204	106921788
RP11-596L14	-0.030806	10	107694481	107873707
RP11-699H2	-0.030806	10	108411165	108583820
RP11-478K18	-0.030806	10	109301761	109497716
RP11-163F15	-0.030806	10	110246657	110410183
RP11-271I13	-0.030806	10	111508658	111672127
RP11-364E8	-0.182928	10	112361447	112508693
RP11-381K7	-0.182928	10	112974154	113145927
RP11-426E5	-0.182928	10	113871121	114065761
RP11-357H24	-0.106844	10	114885414	115067205
RP11-411P18	-0.170779	10	115844971	116003352
RP11-427L15	-0.188532	10	120370659	120567875
RP11-359E7	-0.233664	10	121501561	121679100
RP11-323P17	-0.267136	10	122477477	122477980
RP11-62L18	-0.204763	10	123225110	123397490
RP11-45D20	-0.174421	10	123344229	123515458
RP11-436O19	-0.174421	10	124094555	124277632
RP11-113A6	-0.160888	11	2306063	2473158
RP11-19N21	-0.132046	11	2790044	2962612
RP11-19N21	-0.132046	11	2790044	2962612
RP11-120E20	-0.245995	11	3618360	3800415
RP11-438N5	-0.316319	11	3898611	4048193

FIGURE 2

RP11-309J20	-0.449527	11	4790316	4990480
RP11-364G22	-0.37097	11	5798145	5969718
RP11-89D4	-0.353816	11	7444669	7445456
RP11-379P15	-0.248686	11	8189128	8410723
RP11-152H18	-0.248686	11	8679389	8838111
RP11-243M7	-0.209085	11	9709739	9710446
RP11-327O2	-0.209085	11	10333863	10496425
RP4-607I7	-0.064034	11	35030956	35182961
RP11-472K20	-0.019876	11	35698571	35767187
RP11-115P8	-0.019876	11	35701895	35853383
RP11-324K6	-0.036946	11	37718145	37881309
RP11-307P14	-0.003385	11	40229424	40421930
RP11-100E23	-0.12214	11	51294869	51450781
RP11-163K24	-0.153594	11	62514295	62515049
RP11-231P15	-0.153594	11	62474381	62646249
RP11-138N3	-0.010573	11	67395614	67616066
RP11-569N5	-0.209221	11	68316743	68521009
RP11-804L21	-0.331709	11	69589482	69628306
RP11-21D20	-0.071687	11	70559975	70712757
RP11-598K3	-0.238339	11	70629079	70629611
RP11-512I24	-0.240256	11	70919961	71111150
RP11-31L22	-0.240256	11	72316067	72504103
RP11-93M11	-0.314981	11	72701572	72895150
RP11-28L18	-0.314981	11	74000318	74169974
RP11-28L18	-0.548878	11	74000318	74169974
RP11-167F22	-0.383148	11	74029417	74188898
RP11-535A19	-0.431652	11	75431286	75594844
RP11-25F7	-0.138308	11	76288376	76451702
RP11-25F7	-0.51236	11	76288376	76451702
RP11-30J7	-0.367048	11	76397807	76553748
RP11-321B9	-0.465223	11	77235154	77335154
RP11-321B9	-0.465223	11	77235204	77335204
RP11-7H7	-0.669823	11	78357714	78528143
RP11-153F6	-0.669823	11	78965063	79113170
RP11-187P2	-0.118564	11	80741408	80892446
RP11-90K17	-0.136405	11	85460902	85663450
RP11-320L11	-0.215729	11	86042427	86042949
RP11-137O10	-0.323576	11	87243850	87390553
RP11-49M9	0.481285	11	100108958	100251249
RP11-45C5	0.058039	11	100641187	100793328
RP11-25I9	-0.003696	11	108722050	108887096
RP11-108O10	-0.127936	11	111590961	111769918
RP11-107P10	-0.264486	11	112293289	112467692
RP11-87N22	-0.264486	11	113066688	113257885
RP11-212D19	-0.20639	11	114236228	114372375
RP11-136I14	-0.1532	11	115319527	115487638
RP11-114K7	-0.1532	11	116089651	116251062
RP11-4N9	-0.021098	11	116700206	116862391
RP11-35P15	-0.021098	11	117518653	117688362
RP11-215D10	-0.041798	11	119858549	119859218
RP11-10N17	-0.084891	11	124983596	125140038

FIGURE 2

RP11-100P11	-0.171307	11	125081210	125255316
RP11-432I22	-0.171307	11	126323274	126500239
RP11-106O22	-0.171307	11	126457851	126653816
RP11-168K9	-0.196147	11	126916438	127061799
RP11-264E20	-0.196147	11	128426391	128584603
RP11-567M21	-0.340183	11	129822295	130008331
RP11-567M21	-0.340183	11	129822295	130008331
RP11-340L13	-0.233068	11	131468786	131642985
RP11-419F8	-0.233068	11	132711687	132885208
RP11-545G16	-0.233068	11	132947448	133120135
RP11-149G17	-0.233068	11	134010524	134199934
RP11-469N6	-0.233068	11	134479818	134650277
GS-26-N8	-0.233068	11	134337384	134437384
GS-8-M16	0.161087	12	50000	150000
GS-124-K20	-0.043453	12	50050	150050
RP11-283I3	-0.043453	12	282273	282962
RP11-283I3	-0.043453	12	282273	282962
GS-496-A11	-0.043453	12	665787	765787
RP11-359b12	-0.167774	12	989387	1213323
RP11-359B12	-0.014577	12	989387	1213323
RP11-21K20	-0.014577	12	1850721	1983928
RP5-1096D14	-0.014577	12	1985931	2204029
RP3-461F17	0.090641	12	7139412	7139702
RP11-239A17	0.028678	12	16852561	17016324
RP11-437F6	0.073297	12	23719467	23892262
RP11-707G18	0.073297	12	25379658	25555543
RP11-318G8	0.073297	12	25503820	25664999
RP11-522D14	0.073297	12	25662693	25871054
RP11-350G2	0.006343	12	26604593	26784417
RP11-290I21	0.038341	12	64567977	64568730
RP11-2K12	-0.080029	12	92681767	92842830
RP1-46F2	-0.046075	12	111316270	111463931
RP1-261P5	-0.023583	12	112617924	112753566
RP11-25E2	-0.061054	12	115454531	115641805
RP11-8A1	-0.061054	12	116211328	116380488
RP11-125I23	-0.327073	13	27946897	28110985
RP11-153M24	-0.153731	13	28516250	28516756
RP11-95G6	-0.052618	13	28927701	29071943
RP11-218E6	-0.4846	13	29940843	30109462
RP11-550P23	-0.267297	13	31017797	31149599
RP11-95N14	-0.371997	13	32170305	32341545
RP11-37E23	-0.41993	13	32806968	32971598
RP11-141M1	-0.41993	13	33893732	34048408
RP11-87G1	-0.395778	13	33945471	34126319
RP11-266E6	-0.396421	13	35098112	35260357
RP11-98D3	-0.351275	13	36012940	36181870
RP11-10M8	-0.268906	13	38319921	38320551
RP11-131P10	-0.266521	13	38900297	39052253
RP11-131F1	-0.266521	13	39553249	39709557
RP11-407E23	-0.31579	13	40599391	40600139
RP11-2P5	-0.359128	13	40367434	40566000

FIGURE 2

RP11-125A7	-0.359128	13	42364233	42364987
RP11-117I13	-0.293188	13	43258420	43402191
RP11-168P13	-0.293188	13	44251302	44406457
RP11-442J21	-0.223623	13	45224309	45402217
RP11-351K3	-0.126528	13	46042385	46225345
RP11-408L13	-0.126528	13	48083033	48083775
RP11-305D15	-0.032156	13	48999120	48999915
RP11-185C18	-0.17702	13	50006928	50169848
RP11-40A8	-0.315746	13	51431527	51572831
RP11-327P2	-0.306071	13	52345110	52345932
RP11-431O22	-0.049524	13	53286623	53457898
RP11-384G23	-0.120568	13	53907332	54074848
RP11-319L6	0.017969	13	91545055	91725016
RP11-95C14	0.133857	13	92487444	92653120
RP11-632L2	0.214622	13	93702796	93882311
RP11-62D23	0.253884	13	94432522	94604151
RP11-74A12	0.253884	13	95708902	95709479
RP11-318K19	0.21319	13	96536179	96699791
RP11-235O20	0.21319	13	97346660	97498461
RP11-383H17	0.261407	13	98471162	98668143
RP11-442I9	0.305449	13	100004819	100196340
RP11-279D17	0.305449	13	100320129	100320731
RP11-118F16	0.175092	13	101516431	101681729
RP11-564N10	0.231489	13	102521693	102708370
RP11-255P5	0.231489	13	103461685	103625759
RP11-562E17	0.097007	13	105475125	105643083
RP11-468E2	0.120775	14	24442949	24678228
RP11-89K22	0.186468	14	25479462	25644546
RP11-330O19	0.186468	14	26469450	26686358
RP11-144C18	0.186468	14	27492538	27674153
RP11-419C10	0.168081	14	30187580	30332351
RP11-159L20	0.168081	14	31089528	31254500
RP11-187E13	0.168081	14	32374441	32531022
RP11-501E21	0.168081	14	33310161	33470003
RP11-114L8	0.252033	14	33766983	33928745
RP11-561B11	0.252033	14	35770456	35951901
RP11-259K15	0.197884	14	36544890	36707936
RP11-356O9	0.022831	14	37941646	38143220
RP11-138H18	0.204578	14	38268488	38417016
RP11-506K19	0.204578	14	39279990	39458242
RP11-34O18	0.088668	14	40300279	40467221
RP11-332O9	0.14966	14	41294945	41492454
RP11-168D12	0.14966	14	42272635	42429509
RP11-333K19	0.08548	14	42928208	43100812
RP11-134J10	0.08548	14	44219870	44382366
RP11-99L13	0.08548	14	44838106	44972901
RP11-27P2	-0.435706	14	48816525	48975173
RP11-58E21	-0.001522	14	50538326	50538992
RP11-544I20	-0.309625	14	64273459	64429832
RP11-430G13	-0.309625	14	65129659	65130424
RP11-66E7	-0.262014	14	65958994	66105007

FIGURE 2

RP11-50H7	-0.337473	14	66586729	66754780
RP11-125H8	-0.404435	14	67628639	67799662
RP11-204K16	-0.463173	14	68753875	68924811
RP11-226F19	-0.463173	14	69341229	69505464
RP11-486O13	-0.419185	14	70525284	70729505
RP11-164G17	-0.222319	14	71488026	71488577
RP4-816G1	-0.222319	14	72108120	72237250
RP11-233L14	-0.372673	14	72224012	72270400
RP3-514A23	-0.562377	14	72974679	73155647
RP3-449M8	-0.640902	14	74862252	75000616
RP11-173A8	-0.607955	14	75228320	75408161
RP11-316E14	-0.607955	14	75518904	75519627
RP11-368K8	-0.607955	14	76141892	76325728
RP11-368K8	-0.607955	14	76141892	76325728
RP11-361H10	-0.392555	14	76584595	76762495
RP11-361H10	-0.328973	14	76584595	76762495
RP11-463C8	-0.328973	14	77583617	77584324
RP11-61F4	-0.328973	14	78259832	78442470
RP11-285P21	-0.392422	14	78389382	78593925
RP11-526N18	-0.258069	14	80271909	80450002
RP11-114N19	-0.161968	14	81429028	81587318
RP11-226P1	-0.161968	14	82122485	82305627
RP11-203D9	-0.007058	14	84784824	84930394
RP11-504I1	-0.007058	14	85099070	85099879
RP11-96H16	-0.031253	14	87007447	87008243
RP11-2E15	-0.209689	14	87765087	87915830
RP11-300J18	-0.209689	14	88420711	88596020
RP11-300J18	-0.322333	14	88420711	88596020
RP11-507K2	-0.100872	14	88933009	89112709
RP11-79J20	-0.306695	14	89750111	89916280
RP11-257P13	-0.200003	14	90840977	91024003
RP11-257P13	-0.321917	14	90840977	91024003
RP11-353N19	-0.321917	14	92321034	92490892
RP11-371E8	-0.371428	14	93495784	93496428
RP11-262P9	-0.506966	14	94877177	94877796
RP11-725G5	-0.6878	14	94732236	94885168
RP11-298I23	-0.632062	14	95833001	95996781
RP11-164H13	-0.56186	14	96130887	96294959
RP11-185P18	-0.401862	14	95364095	95527190
RP11-433J8	-0.401862	14	97248817	97249589
RP11-76E12	-0.322019	14	97996975	98163374
RP11-68I8	-0.338241	14	98766862	98940846
RP11-543C4	-0.482754	14	100012907	100176983
RP11-123M6	-0.370752	14	101227262	101390233
RP11-168L7	-0.412084	14	101787803	101975142
RP11-365N19	-0.412084	14	103251182	103408708
RP11-73M18	-0.129459	14	104148594	104312132
RP11-13O24	-0.043714	15	8632	331718
RP11-152F13	0.060857	15	83070992	83226151
RP11-90B9	0.014425	15	83506249	83551620
RP11-365F16	0.014425	15	84215221	84394713

FIGURE 2

RP11-565O12	0.014425	15	85106013	85106544
RP11-356B18	0.027459	15	88671166	88838784
RP3-443N8	0.027959	15	88547448	88647448
RP1-138O23	0.027959	15	88823992	88923992
RP11-405A15	0.011219	15	91506979	91507589
RP11-90E5	0.00282	15	100571562	100756032
GS-124-5	0.021806	15	99988915	100088915
RP11-167B4	-0.049414	16	6226968	6398846
RP11-490O6	-0.032808	16	11888024	11888819
RP11-489O1	-0.091205	16	15505454	15664993
CTD-2504F3	-0.091205	16	16077437	16283248
RP11-378B23	-0.085028	16	16808566	16908566
RP11-429K17	-0.183049	16	20151302	20315827
RP11-489A11	-0.152316	16	21397775	21564932
RP11-101E7	-0.115789	16	22029146	22029952
RP11-105C19	-0.17161	16	22597131	22771241
RP11-105C19	-0.17161	16	22597131	22771241
RP11-548B6	-0.147802	16	23672609	23847849
CTD-2515A14	-0.147802	16	24731063	24947950
RP11-142A12	-0.170707	16	26688287	26819858
RP11-142A12	-0.009298	16	26688287	26819858
F21283	-0.137249	16	28604078	28704078
RP11-74E23	-0.107586	16	29644284	29822033
RP11-2C24	-0.107586	16	30633463	30839321
RP11-388M20	-0.018326	16	31048088	31282150
RP11-5L1	0.091194	16	46510743	46672574
RP11-283C7	0.117577	16	46946360	47129544
RP11-283C7	0.117577	16	46946360	47129544
RP11-523L20	0.127342	16	47889993	48046856
RP11-452G23	0.619446	16	48682426	48839286
RP11-452G23	0.558504	16	48682426	48839286
RP11-147B17	0.363561	16	50525390	50694156
RP11-424K7	0.610472	16	51061729	51235705
RP11-122K22	0.610472	16	51873878	51874527
RP11-467J12	0.610472	16	52909551	53111596
RP11-357N13	0.148594	16	53871620	54067953
RP11-357N13	0.258625	16	53871620	54067953
RP11-7O2	0.339914	16	55267280	55417386
RP11-165M2	0.076808	16	55869021	56001451
RP11-325K4	0.248781	16	56813097	57000794
RP11-405F3	0.103068	16	57622904	57772978
RP11-481J2	0.024436	16	58395492	58581463
RP11-481J2	0.024436	16	58395492	58581463
RP11-457D20	0.171614	16	60220086	60434930
RP11-157H19	0.07654	16	61801773	61802422
RP11-467O15	0.152921	16	62541767	62728484
RP11-148F12	0.155872	16	63387399	63522174
RP11-370P15	0.151156	16	63838775	63839447
RP11-298C15	0.028886	16	65378281	65583547
RP11-298C15	0.028886	16	65378281	65583547
RP11-311C24	-0.108689	16	69607426	69608066

FIGURE 2

RP11-296I10	-0.414787	16	70133673	70312186
RP11-296I10	-0.305336	16	70133673	70312186
RP11-556H2	-0.009703	16	79043072	79223295
RP1-27J12	0.017801	17	14423818	14424490
RP11-64B12	0.018149	17	14593233	14657803
RP11-385D13	0.087973	17	15381603	15568444
RP11-219A15	0.196775	17	16587021	16777061
RP11-524F11	0.198667	17	17398429	17579737
RP11-189D22	0.151592	17	18000668	18172954
CTB-1187M2	0.024885	17	19272101	19316085
RP11-78O7	0.246235	17	19674485	19807021
RP5-836L9	0.042467	17	20073751	20192547
RP11-121A13	0.068523	17	20172437	20288709
RP11-64J19	-0.001939	17	21055083	21191541
RP11-260A9	0.304594	17	25440972	25441520
RP11-229K15	0.368536	17	29271460	29427884
RP11-142O6	0.285307	17	29550205	29550969
RP11-474K4	0.146122	17	30393209	30570951
RP11-215E13	-0.133727	17	32489785	32642786
RP5-837J1	0.034409	17	33317034	33416269
RP11-47L3	0.490027	17	33500468	33660812
RP11-445F12	0.4177	17	35116399	35296332
RP11-94L15	-0.137472	17	37812853	37972567
RP5-1110E20	0.528741	17	38984203	39119156
RP11-156E6	0.572504	17	39940099	40116322
RP11-506G7	0.572504	17	40884763	41073084
RP11-376M2	0.572504	17	40968824	41171464
RP11-948G15	0.572504	17	41171833	41172398
RP11-392O1	0.572504	17	41572478	41735005
RP5-905N1	0.156688	17	41826877	41827638
RP5-1169K15	0.107933	17	43339849	43430323
RP5-843B9	0.107933	17	44084882	44094836
RP5-971F3	0.210633	17	41818827	41939592
RP11-506D12	-0.045637	17	48817562	49024474
RP11-217N19	-0.019738	17	52399470	52566958
RP11-283E7	-0.012275	17	66771269	66941683
RP11-166M16	0.024014	17	69658425	69814539
RP11-478P5	0.034421	17	72268412	72462136
RP11-141D15	-0.009074	17	76125635	76301246
GS-52-M11	-0.251919	18	0	100000
GS-74-G18	-0.251919	18	162949	262949
RP11-324G2	-0.251919	18	179428	349445
RP11-267C19	-0.039286	18	967589	968333
RP11-308J14	-0.094755	18	8806684	8982445
RP11-99M10	-0.043828	18	10447604	10596116
RP11-164M8	0.114787	18	39052514	39227568
RP11-748M14	0.037744	18	45336521	45490381
CTD-3149D2	-0.06166	19	17701434	17925502
RP11-943H6	-0.229612	19	17702637	17802637
RP11-165F1	0.111524	19	22342884	22496031
CTD-2579N5	0.201129	19	22984141	23157068

FIGURE 2

RP11-359H18	0.203608	19	23764233	23939462
RP11-521I20	-0.009738	19	49834840	50025458
CTD-2545M3	0.27721	19	50897959	51060255
GS-82-O2	0.195473	20	130000	230000
RP5-852M4	-0.036076	20	328014	475783
RP11-314N13	-0.036076	20	1181792	1371966
RP5-1140M3	-0.285629	20	8209614	8379068
RP4-811H13	-0.285629	20	9250717	9391955
RP11-204H22	-0.071623	20	10451757	10633167
RP4-742J24	-0.071623	20	11865380	12030405
RP11-234K24	-0.062688	20	34706346	34882247
RP3-470L14	-0.140544	20	47636060	47809221
RP4-715N11	0.010711	20	51284969	51394314
RP11-304D2	0.154417	21	19303137	19461093
RP11-509A1	0.197586	21	22203093	22354855
RP1-50A12	0.197586	21	22316309	22488461
RP11-258A5	0.039774	21	23231053	23410836
RP11-15H23	0.039774	21	25587202	25587712
RP1-255P7	0.039732	21	36361439	36609975
RP11-155F20	-0.148898	22	19310306	19458514
RP11-80O7	-0.201505	22	24181132	24458754
LL22NC03-95F10	-0.179077	22	25057726	25058244
CTA-221G9	-0.179077	22	23879817	23982359
CTA-125H2	-0.179077	22	24560701	24734213
CTA-445C9	-0.179077	22	25228499	25359896
RP3-353E16	-0.218972	22	28168322	28391737
RP11-329J7	-0.15257	22	28774294	28986685
CTA-57G9	-0.15257	22	27842535	27956406
RP4-539M6	-0.191813	22	30788544	30978289
RP3-515N1	-0.359607	22	31568783	31701831
RP1-127L4	-0.233942	22	30835421	30883232
XXbac-677f7	-0.233942	22	33169418	33296100
CTA-415G2	-0.142924	22	31695938	31825894
LL22NC01-132D12	-0.004841	22	35478908	35505203
CTA-390B3	-0.058909	22	36120349	36212755
CTA-228A9	-0.150749	22	38482376	38575714
CTA-150C2	-0.150749	22	39281250	39480329
LL22NC03-10C3	-0.288987	22	37938484	37976499
RP1-172B20	-0.161693	22	40016508	40228143
RP3-388M5	-0.019282	22	42473525	42651092
GS-839-D20	0.428842	23	110000	210000
GS-98-C4	0.643112	23	440000	540000
RP11-418N20	0.112672	23	2930526	3111894
RP11-23N11	0.062576	23	3707732	3754886
RP11-483M24	0.210165	23	6948788	7125832
RP11-323F16	0.076683	23	7446566	7622822
RP1-108M6	0.015246	23	9601995	9723171
RP6-1O2	0.015246	23	10308319	10476613
RP11-1J4	0.273793	23	12540561	12632714
RP11-1J4	0.171176	23	12540561	12632714
RP1-164K3	0.13605	23	13043422	13147981

FIGURE 2

RP1-122K4	0.13605	23	13906016	14104117
RP1-93D11	0.13605	23	14852690	15010369
RP11-431J24	0.042102	23	16119734	16331718
RP5-1129A6	0.09684	23	18172945	18317643
RP11-558P14	0.09684	23	18365515	18542396
RP11-421K1	0.09684	23	19245771	19246392
RP11-406A18	0.154731	23	21539344	21690173
RP11-261M11	0.192769	23	24268031	24430811
RP11-272N24	0.192769	23	25337706	25338461
RP11-26L4	0.216937	23	26894928	27060256
RP11-489K4	0.051206	23	28120950	28276180
RP11-37E19	0.128159	23	29018586	29019209
RP6-27C10	0.128159	23	29102825	29232829
RP4-662N3	0.201728	23	29593002	29593694
RP11-122N14	0.201728	23	31518157	31679330
RP4-639D23	0.041451	23	32233440	32366887
RP5-1147O16	0.041451	23	32438265	32568505
RP11-12J5	0.085112	23	37669959	37830488
RP11-12J5	0.085112	23	37669959	37830488
RP1-169I5	0.130957	23	41107931	41241498
RP5-879N19	0.086982	23	43468696	43563277
RP11-576G22	0.102115	23	45256642	45434291
RP11-253L3	-0.020798	23	73471329	73638506
RP3-380C13	0.03489	23	94692551	94829928
RP11-274M8	0.054965	23	96464293	96621678
RP5-965E19	0.129764	23	131357146	131514495
RP6-198C21	0.129764	23	132395417	132536303
RP11-481F23	0.440201	23	134603533	134803789
RP1-196E23	0.287047	23	135361435	135484339
RP5-833B2	0.245947	23	137995743	138080362
RP13-34G21	0.245947	23	138760670	138948618
RP4-595A18	0.245947	23	139603351	139745257
RP11-518F7	0.27191	23	140385198	140548576
RP4-552K20	0.450723	23	140891246	140891811
RP3-324C6	0.182749	23	141533809	141648115
GS1-91O18	0.120938	23	142294977	142411207
RP1-29A6	0.282421	23	143614272	143617166
RP11-390O24	0.33793	23	144177088	144353695
RP11-387H19	0.297334	23	145042542	145222156
RP1-73A14	0.229363	23	145778695	145884842
RP3-433G13	0.320371	23	146664917	146721650
RP6-224C24	0.320371	23	146697323	146873742
RP5-892C22	0.043261	23	150436146	150546295
RP11-54I20	0.145657	23	152707082	152884336
RP11-54I20	0.259044	23	152707082	152884336
RP5-1087L19	0.151835	23	153752486	153893678
RP11-296N8	0.072446	23	154131702	154308447
RP11-218L14	0.009194	23	154682277	154828136