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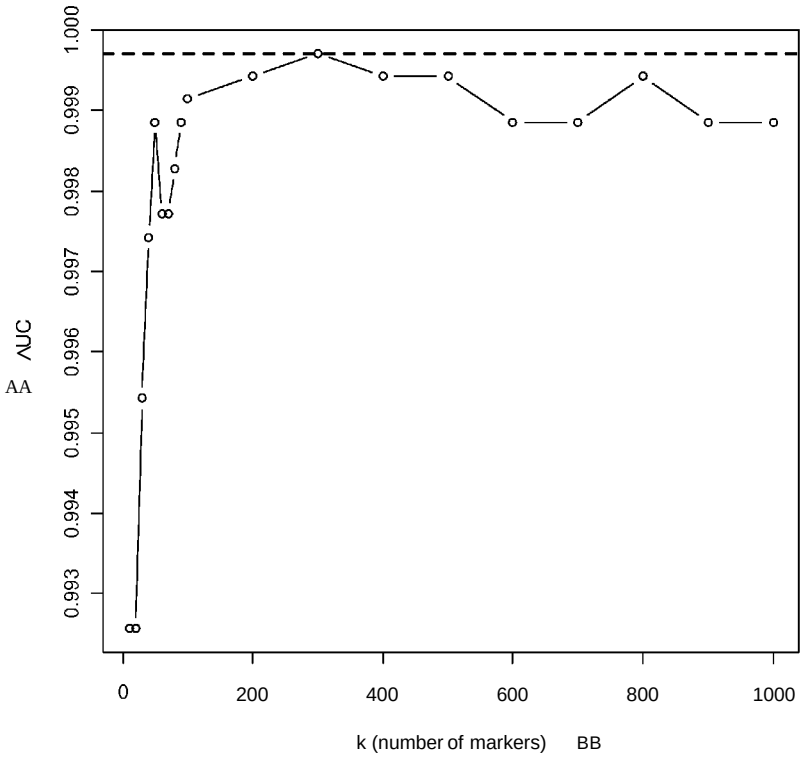
(54) Title: METHYLATION BIOMARKERS FOR OVARIAN CANCER

(57) Abstract: The present disclosure provides differentially methylated gen- omic CpG dinucleotide sequences asso- ciated with cancer. In particular, differ- entially methylated genomic CpG nuc- leotides and their use in diagnostic and prognostic methods for ovarian cancer are disclosed.

FIGURE 2



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METHYLATION BIOMARKERS FOR OVARIAN CANCER

The present application claims priority to United States provisional patent application serial no. 61/579,537 filed 22 December 2011 which is incorporated herein by reference in its entirety.

BACKGROUND

Ovarian cancer is the among the top ten most common cancers among women, and the fifth leading cause of death for women with cancer in the United States. Worldwide, the most lethal gynecological disease among women in developed countries is ovarian cancer. The American Cancer Society estimates that about 22,000 new cases will be diagnosed this year and approximately 15,000 women will die from ovarian cancer in the United States alone. The incidence rate of ovarian cancer is roughly 13 per 100,000 women per year and even though the median age at diagnosis is around 63, no age group is immune to the disease. Further, even though incidence of ovarian cancer is slightly higher among white women, no race or ethnic background is immune.

Survival rate once a diagnosis is made is typically dismal, for example if ovarian cancer is detected and effectively treated prior to metastasis the 5 year survival rate can be as high as 73%, however if the cancer is not detected until it has metastasized then the long term survival rate drops to <30%. Unfortunately, ovarian cancer often goes undetected until it has metastasized within the pelvis and abdomen, therefore the outcome for most women is grim as the cancer is difficult to treat and is often fatal.

As such, early detection ovarian cancer is crucial to benefit those patients, for example, that present with no or vague symptoms or with tumors that are below the level of detection during a physical examination. A considerable amount of research effort has been focused in discovering and developing early detection systems, however to date no effective screening method has been developed. As such, what are needed are ways to detect ovarian cancer, preferably at an early stage, for example

before metastasis, thereby improving the long term survival of those women afflicted with this disease.

Changes in cellular genetic information, such as mutations in gene sequences which can affect gene expression and/or protein sequence, are associated with many diseases and cancers. However, changes can also occur to genes that affect gene expression; changes caused by mechanisms other than genetic mutations. Epigenetics is the study of changes in gene expression caused by mechanisms other than changes in the underlying DNA sequence, the methylation of DNA being one of those mechanisms. Methylation of DNA, the addition of a methyl group to the 5 position of a cytosine pyrimidine ring or the positional sixth nitrogen of an adenine purine ring, is widespread and plays a critical role in the regulation of gene expression in development and differentiation of diseases such as multiple sclerosis, diabetes, schizophrenia, aging, and cancers. In adult somatic cells, DNA methylation typically occurs in regions where a cytosine nucleotide (C) is found next to a guanine nucleotide (G) where the C and G are linked by a phosphate group (p), the linear construct being referred to as a "CpG" island. Methylation in particular gene regions, for example in gene promoter regions, can augment or inhibit the expression of these genes. Recent work has shown that the gene silencing effect of methylated regions is accomplished through the interaction of methylcytosine binding proteins with structural components of chromatin which, in turn, makes the DNA inaccessible to transcription factors through histone deacetylation and chromatin structure changes.

Changes in DNA methylation have been recognized as one of the most common molecular alterations in human neoplasia. Hypermethylation of CpG islands located in promoter regions of tumor suppressor genes is firmly established as the most frequent mechanism for gene inactivation in cancers. In contrast, hypomethylation of genomic DNA are observed in tumor cells. Further, a correlation between hypomethylation and increased gene expression has been reported for many oncogenes. Monitoring global changes in methylation pattern has been applied to molecular classification of cancers, for example, gene hypermethylation has been associated with clinical risk groups in neuroblastoma and hormone receptor status correlation with response to tamoxifen in breast cancer.

SUMMARY

The present disclosure identifies biological markers, or biomarkers, indicative of ovarian cancer. The biomarkers and methods of their use described herein provide alternatives to currently available ovarian cancer determinative, diagnostic and prognostic methodologies. Biomarkers and methods of their use can also be used to assess an individual prior to, during and/or subsequent to a treatment course for eradicating ovarian cancer (e.g., chemotherapy, radiation therapy, drug therapy, etc.) for determining the effectiveness of a treatment course.

The present disclosure provides methods for identifying differentially methylated genomic CpG dinucleotide sequences associated with ovarian cancer in an individual comprising measuring the level of methylation in a biological test sample from an individual suspected of having ovarian cancer at a CpG dinucleotide sequence in one or more genomic targets of SEQ ID NOs: 1-300, comparing the level of methylation at the one or more CpG dinucleotide sequences in said biological test sample to a reference level of methylation of said genomic CpG dinucleotide sequences in a normal biological sample wherein a difference in the methylation levels between the test sample and the normal sample identifies differentially methylated genomic CpG dinucleotide sequences associated with ovarian cancer in said individual. In some embodiments, the level of methylation of the differentially methylated genomic CpG dinucleotide sequence is used to diagnose ovarian cancer in said individual. In some embodiments, the methylation level in a biological sample is decreased as compared to a reference sample level, whereas in other embodiments the methylation level in a biological sample is increased relative to a control reference sample.

In some embodiments, the level of methylation in the biological sample is decreased in SEQ ID NOs: 1-2, 10, 13-14, 17-18, 21, 28, 30-31, 34, 41-42, 46, 51, 54-58, 63, 65-66, 73, 80-81, 83, 88, 91-96, 98-99, 104-105, 110, 115-116, 125-126, 130-131, 133, 136, 138-139, 143-145, 147-150, 153, 157, 159-160, 163, 166, 168-169, 171, 175, 178-180, 183, 185, 187-189, 197, 200, 202, 204, 212-213, 215-216, 223-225, 231-233, 239, 241, 245-247, 250-252, 254, 256-260, 264-266, 268, 271-272, 274, 276-277, 280-287, 289, 290, 292, 297 and 299-300 in comparison to the reference level, whereas in other embodiments the level of methylation in the

biological sample is increased in SEQ ID NOs: 3-9, 11-12, 15-16, 19-20, 22-27, 29, 32-33, 35-40, 43-45, 47-50, 52-53, 59-62, 64, 67-72, 74-79, 82, 84-87, 89-90, 97, 100-103, 106-109, 111-114, 117-124, 127-129, 432, 134-135, 137, 140-142, 146, 151-152, 154-156, 158, 161-162, 164-165, 167, 170, 172-174, 176-177, 181-182, 184,186, 190-196, 198-199, 201, 203, 205-211, 214, 217-222, 226-230, 234-238, 240, 242-244, 148-149, 253, 255, 261-263, 267, 269-270, 273, 275, 278-279, 288, 291, 293-296 and 298 in comparison to the reference level.

In some embodiments, the differentially methylated genomic CpG dinucleotide sequences are observed in a subset of said genomic targets, wherein the subset comprises two or more of the genomic targets set forth as SEQ ID NOS: 200, 58, 41, 153, 57, 274, 168, 223, 197, 18, 289, 266, 26, 60, 190, 122, 112, 111, 22, 151, 146, 251, 104, 72, 6, 62, 33, 277, 204, 110, 42, 113, 165, 132, 212, 179, 128, 167, 238, 103, 295, 69, 74, 156, 77, 43, 102, 61, 234, 100, 27, 244, 89, 82, 275, 255, 230, 211, 79, 85, 263, 121, 15, 243, 172, 154, 71, 86, 228, 164, 134, 235, 174, 38, 222, 269, 70, 123, 45, 36, 87, 49, 226, 84, 166, 34, 125, 265, 144, 63, 66, 285, 163, 260, 270, 209, 127, 152, 93, 133, 253, 114, 11, 9, 4, 7, 7, 107, 12, 3, 20, 5, 117, 186, 205 and 206.

In some embodiments, genomic CpG dinucleotides indicative of ovarian cancer comprises a population of genomic targets comprising SEQ ID NOS: 1-300 or a subset of said population. In some embodiments, the population targets are capable of exhibiting differential methylation of genomic CpG dinucleotide sequences, wherein said differential methylation is diagnostic for the presence of ovarian cancer in an individual. In some embodiments, the population of genomic targets comprises a subset of SEQ ID NOS: SEQ ID NOS: 200, 58, 41, 153, 57, 274, 168, 223, 197, 18, 289, 266, 26, 60, 190, 122, 112, 111, 22, 151, 146, 251, 104, 72, 6, 62, 33, 277, 204, 110, 42, 113, 165, 132, 212, 179, 128, 167, 238, 103, 295, 69, 74, 156, 77, 43, 102, 61, 234, 100, 27, 244, 89, 82, 275, 255, 230, 211, 79, 85, 263, 121, 15, 243, 172, 154, 71, 86, 228, 164, 134, 235, 174, 38, 222, 269, 70, 123, 45, 36, 87, 49, 226, 84, 166, 34, 125, 265, 144, 63, 66, 285, 163, 260, 270, 209, 127, 152, 93, 133, 253, 114, 11, 9, 4, 7, 7, 107, 12, 3, 20, 5, 117, 186, 205 and 206.

In some embodiments, differential methylation in a population of genomic targets is decreased methylation level of said CpG dinucleotide sequences in a sample

from an individual in comparison to a reference level, for example as found in SEQ ID NOs: 1-2, 10, 13-14, 17-18, 21, 28, 30-31, 34, 41-42, 46, 51, 54-58, 63, 65-66, 73, 80-81, 83, 88, 91-96, 98-99, 104-105, 110, 115-116, 125-126, 130-131, 133, 136, 138-139, 143-145, 147-150, 153, 157, 159-160, 163, 166, 168-169, 171, 175, 178-180, 183, 185, 187-189, 197, 200, 202, 204, 212-213, 215-216, 223-225, 231-233, 239, 241, 245-247, 250-252, 254, 256-260, 264-266, 268, 271-272, 274, 276-277, 280-287, 289, 290, 292, 297 and 299-300 in comparison to a reference level. In some embodiments, the differential methylation of a population of genomic targets is increased methylation level of said CpG dinucleotide sequences in a sample from an individual in comparison to a reference level, for example as found in SEQ ID NOs: 3-9, 11-12, 15-16, 19-20, 22-27, 29, 32-33, 35-40, 43-45, 47-50, 52-53, 59-62, 64, 67-72, 74-79, 82, 84-87, 89-90, 97, 100-103, 106-109, 111-114, 117-124, 127-129, 432, 134-135, 137, 140-142, 146, 151-152, 154-156, 158, 161-162, 164-165, 167, 170, 172-174, 176-177, 181-182, 184,186, 190-196, 198-199, 201, 203, 205-21 1, 214, 217-222, 226-230, 234-238, 240, 242-244, 148-149, 253, 255, 261-263, 267, 269-270, 273, 275, 278-279, 288, 291, 293-296 and 298 in comparison to a reference level.

FIGURES

Figure 1 is an exemplary receiver-operator characteristic (ROC) curve for the subset of DNA methylation markers (1-100) of Figure 1. Data used for the graph are distinct from the data used to select the markers.

Figure 2 demonstrates the specificity of cumulative subsets of DNA methylation markers. The smaller circles represent cumulative subsets of n = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 markers moving from left to right. The large, bolded circle represents the area under the curve (AUC) maximized at 300 markers. Data used for the graph are distinct from the data used to select the markers.

Figure 3 shows a series of box plot graphs from subsets of markers (k) of Figure 1. The graphs demonstrate a comparison between the percentage of markers that have β values greater/less than the threshold defined for that marker (y axis) between the ovarian tumor formaldehyde fixed, paraffin embedded (FFPE) tissue

samples, normal ovarian tissue samples and normal non-ovarian tissue samples (Other Tissue). The box graph representing 10 markers corresponds to the first 10 markers (k=10) of Figure 2, 50 markers corresponds to the first 50 markers (k=50), 100 markers corresponds to the first 100 markers (k=100), 200 markers corresponds to the first 200 markers (k=200) and 300 markers corresponds to the first 300 markers (k=300). Data used for the graph are distinct from the data used to select the markers.

DEFINITIONS

As used herein, the term "sample" is intended to mean any biological fluid, cell, tissue, organ or portion thereof that contains genomic nucleic acids, for example genomic DNA, suitable for methylation status determination via the disclosed methods. A test sample can include or be suspected to include a cell, such as a cell from an ovary, uterus, fallopian tube, vagina, or other organ or tissue that contains or is suspected to contain a cancerous cell. The term includes samples present from an individual as well as samples obtained or derived from an individual. For example, a sample can be a histologic section of a specimen obtained by biopsy, cell scraping, etc. or cells that are placed in or adapted to tissue culture. A sample further can be a sub-cellular fraction or extract, or a crude or isolated nucleic acid molecule. A sample further can be a serum or other fluidic sample suspected of containing circulating cells. A normal sample can be used to establish a methylation background pattern for comparison to a test sample.

A sample may be obtained in a variety of ways known in the art. Samples may be obtained according to standard techniques from all types of biological sources that are usual sources of genomic DNA including, but not limited to cells or cellular components which contain DNA, cell lines, circulating tumor cells, biopsies, bodily fluids such as blood, lavage specimens, tissue samples such as tissue that are formalin fixed and embedded in paraffin such as tissue from ovaries, endometrium, cervix, fallopian tubes, omentum, histological object slides, and all possible combinations thereof. Further, tissues can be fresh, fresh frozen, etc. Accordingly, a sample can be from an archived, stored or fresh source as suits a particular application of the methods set forth herein. In particular embodiments, the methods described herein can be performed on one or more samples from ovarian cancer patients such as samples

obtained by vaginal lavage, endometrial biopsy, ovarian biopsy, and/or blood draw. Sample analysis can be applied, for example, to determine the methylation status of cells suspected of being ovarian cancer cells, to determine the methylation status of cells for differentiation between early and/or late stage ovarian cancer types, ovarian cancer epithelial type differentiation, or to determine the methylation status of cells in order to monitor cancer progression or response to treatment.

A suitable sample can be collected and acquired that is either known to comprise ovarian cancer cells or is subsequent to the formulation of the diagnosis of ovarian cancer. A sample can be derived from a population of cells or from a tissue that is predicted to be afflicted with, or phenotypic of, ovarian cancer. The genomic DNA can be derived from a high-quality source such that the sample contains only the tissue type of interest, minimum contamination and minimum DNA fragmentation. In particular, samples are contemplated to be representative of the tissue or cell type of interest that is to be handled by an assay. In addition, a population or set of samples from an individual source can be analyzed to maximize confidence in the results for an individual. In some embodiments, a sample from an individual is matched and compared to a normal sample from that same individual to identify the DNA methylation status of certain CpG dinucleotides for that individual. A normal sample, such as a patient matched normal sample, can be from the same or similar organ, tissue or fluid as the sample to which it is compared. The normal sample will typically display a phenotype that is different from a phenotype of the sample to which it is compared.

As used herein, the term "isolated" or "purified" when used in relation to a nucleic acid refers to a nucleic acid sequence that is extracted and separated from at least one component or contaminant with which it is ordinarily associated in its natural source. As such, an isolated or purified nucleic acid is present in a form or setting that is different from that in which it is found in nature.

The term "gene" refers to a nucleic acid sequence, such as DNA, that comprises coding sequences associated with the production of a polypeptide, precursor, or RNA (e.g., rRNA, tRNA). A gene also includes non-coding and intergenic sequences. The term can encompass the coding region of a gene and the sequences located adjacent to the coding region on both the 5' and 3' such that the

gene corresponds to the length of the full-length mRNA. Sequences located 5' of the coding region and present on the mRNA are referred to as 5' non-translated sequences and comprise promoter, enhancer, transcription factor binding sites, and the like which affect gene expression. Sequences located 3' or downstream of the coding region and present on the mRNA are referred to as 3' non-translated sequences. The term "gene" encompasses both cDNA and genomic forms of a gene. A genomic form or clone of a gene contains the coding region interrupted with non-coding sequences such as introns, intervening regions, intervening sequences or intergenic regions. Conversely, a "non-gene associated region" are those genetic regions in a genome that are currently not identified as being related to a particular gene as identified above.

As used herein, the term "reference level" refers to a control level of a marker used to evaluate a test level of a biomarker in a sample of a patient. For example, the level of methylation of one or more CpG dinucleotides, in a gene region or a non-gene associated region, in a patient sample can be higher than the reference level, wherein the cells are considered to have a higher level of methylation as compared to the methylation level found in a normal reference cell sample. Conversely, the level of methylation of one or more CpG dinucleotides in the test cells of a patient can be lower than the reference level, wherein the test cells are considered to have a lower level of methylation relative to the normal cell sample.

A reference level can be determined based on samples collected from normal classes of adjacent tissues and/or with normal peripheral blood lymphocytes. The reference level can be determined by any of a variety of methods. The reference level can be determined by, for example, measuring the level of methylation of a biomarker in non-tumorous cells from the same tissue as the tissue of the neoplastic cells to be tested. The reference level can also be a level of a biomarker of in vitro cultured cells which can be manipulated to yield methylation levels which accurately reflect methylation levels of a normal cell or tissue sample. The reference level can also be determined by comparison of the level of a biomarker, such as methylation of one or more genes, in populations of patients having the same cancer. This can be accomplished, for example, by histogram analysis, in which an entire cohort of patients are graphically presented, wherein a first axis represents the level of the

biomarker, and a second axis represents the number of patients in the cohort whose neoplastic cells express the biomarker at a given level.

Two or more separate groups of patients can be determined by identification of subset populations of the cohort which have the same or similar levels of the biomarker. Determination of the reference level can then be made based on a level which best distinguishes these separate groups. A reference level also can represent the levels of two or more markers. Two or more markers can be represented, for example, by a ratio of values for levels of each biomarker. The reference level can be a single number, equally applicable to every patient, or the reference level can vary, according to specific subpopulations of patients. For example, individuals with a certain subtype of cancer might have a different reference level than individuals of a different subtype of cancer, say subtypes of ovarian cancer. In another example, the reference level might be a certain ratio of a biomarker in the neoplastic cells of a patient relative to the biomarker levels in non-tumor cells within the same patient. Thus the reference level for each patient can be proscribed by a reference ratio of one or more genomic markers, such as methylation of one or more genes or non-gene related CpG dinucleotides, wherein the reference ratio can be determined by any of the methods for determining the reference levels described herein.

It is understood that the reference level corresponds to the level of one or more methylated genomic CpG dinucleotide sequences present in a corresponding sample that allows comparison to the desired phenotype. For example, in a diagnostic application a reference level can be based on a sample that is derived from a cancer-free origin so as to allow comparison to the biological test sample for purposes of diagnosis. In a method of staging a cancer it can be useful to apply in parallel a series of reference levels, each based on a sample that is derived from a cancer that has been classified based on parameters established in the art, for example, phenotypic or cytological characteristics, as representing a particular cancer stage so as to allow comparison to the biological test sample for purposes of staging. In addition, progression of the course of a condition can be determined by determining the rate of change in the level or pattern of methylation of genomic CpG dinucleotide sequences by comparison to reference levels derived from reference samples that represent time points within an established progression rate. A user will be able to select the

reference sample and establish the reference level based on the particular purpose of the comparison.

As used herein, the term "neoplastic cell" refers to any cell that is transformed such that it proliferates without normal homeostatic growth control, for example a cancer cell, in particular an ovarian cancer cell. Such cells can result in a benign or malignant lesion of proliferating cells. Such a lesion can be located in a variety of tissues and organs of the body, in particular from female reproductive tissues. The term "cancer" is intended to mean a class of diseases characterized by the uncontrolled growth of aberrant cells, including all known cancers, and neoplastic conditions, whether characterized as malignant, benign, soft tissue or solid tumor.

As used herein, the term "disease-free survival" refers to the lack of tumor recurrence and/or spread and the fate of a patient after diagnosis, for example, a patient who is alive without tumor recurrence.

The phrase "overall survival" refers to the fate of the patient after diagnosis, regardless of whether the patient has a recurrence of the tumor. As used herein, the term "risk of recurrence" refers to the probability of tumor recurrence or spread in a patient subsequent to diagnosis of cancer, wherein the probability is determined according to the process of the invention. Tumor recurrence refers to further growth of neoplastic or cancerous cells after diagnosis of cancer. Particularly, recurrence can occur when further cancerous cell growth occurs in the cancerous tissue. Tumor spread refers to dissemination of cancer cells into local or distant tissues and organs, for example during tumor metastasis. Tumor recurrence, in particular, metastasis, is a significant cause of mortality among patients who have undergone surgical treatment for cancer. Therefore, tumor recurrence or spread is correlated with disease free and overall patient survival.

DETAILED DESCRIPTION

Ovarian cancer is often referred to as a silent killer because of its subtle symptoms that lead to delayed discovery, diagnosis and treatment. The majority of ovarian cancers are diagnosed when the cancer has already reached an advanced

stage, for example >80% of serous ovarian cancers are diagnosed at Stage III or Stage IV leading to a very low chance of long-term survival in these patients. Screening and/or detecting ovarian cancer in women who might be at higher risk of developing ovarian cancer, such as those with a strong family history of such cancer, is problematic. The two most common screening tests for ovarian cancer include transvaginal sonography and identification of a protein marker, CA-125. However, both tests have limitations. For example, transvaginal sonography can identify a mass in the ovary however the sonogram is unable to distinguish whether the mass is cancerous or not. The protein marker CA-125 is not specific to the presence of ovarian cancer as other cancers also exhibit high levels of CA-125.

The majority of ovarian tumor cancers are of the epithelial histologic type, which can be further divided into different tumor subtypes, for example serous, endometrioid, clear cell, mucinous, Brenner or transitional cell, squamous cell, undifferentiated and mixed epithelial cell types (AJCC Cancer Staging Manual 7th Ed., p.422). There are several different methods for grading or staging cancers. Perhaps the most clinically applied is the tumor node metastasis (TNM) staging system and/or the staging system as described by the Federation Internationale de Gynecologie et d'Obstetrique (FIGO).

Ovarian cancer can also be classified into two groups based on molecular progression. For example, Type I ovarian tumors of mucinous, clear cell, endometrioid, and low-grade serous type develop in stepwise fashion from adenomas to carcinomas, whereas Type II tumors of high-grade serous develop *de novo* from undefined precursor lesions and progress rapidly with no apparent stepwise progression (Ie and Kurman, 2004, Am J Pathol 164:151 1-151 8). Further explanation of cancer staging and grading can be found at, for example, AJCC Cancer Staging Manual, Edge, SB et al, Eds., Springer-Verlag, New York. The vast majority of Type II, high-grade serous ovarian cancers (OvCa) are diagnosed at advanced stages and represent a major challenge in early detection (Chan et al, 2006, Obs and Gyn 108: 521-528).

The most common type of ovarian cancer arises from epithelial cells that line the surface of the ovary. Approximately 50% of epithelial ovarian tumors are classified as serous, or tumors with glandular features, and make up approximately

80% of all ovarian tumors. Other types of ovarian cancers can arise from germ cells (e.g., cancer of the ovarian egg-making cells) and sarcomas. High-grade serous tumors denote highly aggressive, invasive tumors as compared to low malignant potential (LMP) tumors. Whether an invasive serous tumor is classified as either high or low grade is based on the clinical course of the disease. For example, high grade serous tumors were found to over express genes that control various cellular functions associated with cancer cells, for example genes that control cell growth, DNA stability (or lack thereof) and genes that silence other genes. Conversely, LMP tumors were not found to overexpress these types of genes and LMP tumors were alternatively characterized by expression of growth control pathways, such as tumor protein 53 (TP53 or p53) pathways.

More recently, a two-tiered system of characterizing serous ovarian tumors has been described (Vang, et al, 2009, Adv Anat Pathol 16:267-282) based on studies performed Johns Hopkins Hospital and M.D. Anderson Cancer Center. Briefly, low grade serous ovarian tumors are characterized based in a number of criteria, for example low grade serous tumors have low to no chromosomal instability, typically have mutated KRAS, BRAF and ERBB2 genes, demonstrate slow tumor development, typically have cell nuclei that are uniform, small and round and generally have low mitotic index. Conversely, a high grade serous tumor has a high degree of chromosomal instability, has mutated TP53 gene, demonstrates very fast tumor development, typically has nuclei that are non-uniform, enlarged and irregularly shaped and has high mitotic index.

However, staging and grading cancers are subjective and rely on a diagnostician to interpret morphology, histology, anatomy and other related indices. Further, as ovarian cancer is typically left undiagnosed until late stage cancer due to, for example, its asymptomatic phenotype, the staging and grading do nothing to identify early stage cancer, or identify ovarian cancer earlier in the disease progression in the absence of disease related symptoms. As such, there is a critical need for tools, methods and strategies that can be used for detecting, diagnosing, and prognosing ovarian cancer in a patient.

DNA methylation is a cancer type-specific epigenetic event that plays an important role in tumor development and the identification of cancer-specific

epigenetic changes has promise as a potential tool in molecular classification and disease stratification. Experiments conducted with regards to determining epigenetic changes associated with ovarian cancer have resulted in the identification of aberrant DNA methylation that is present in ovarian cancer. Assays to determine the aberrant DNA methylation patterns, or DNA methylation biomarkers, as defined herein can be performed not only on cells, but also on DNA from circulating tumor cells in the blood thereby providing an assay for ovarian cancer that is non-invasive.

The present disclosure describes embodiments directed to diagnostic and prognostic compositions and methods for identifying epigenetic modifications present in ovarian cancer cellular DNA. In particular, embodiments disclosed herein characterize ovarian cancer by differential methylation of genomic CpG dinucleotide sequences and provide populations of genomic targets useful for detecting the differential methylation of genomic CpG dinucleotide sequences in diagnosing an individual with ovarian cancer, determining the prognosis of an individual identified with ovarian cancer, and/or treatment assessment options for an individual afflicted with ovarian cancer.

Experiments were performed on samples from patient cohorts to identify genomic CpG dinucleotide sequences exhibiting variant methylation as compared to a reference or normal control sample. Those CpG dinucleotide sequences that were differentially methylated as compared to a reference sequence are recognized herein as biomarkers for detecting, diagnosing, prognosing or assessing a patient with ovarian cancer. From the patient cohort samples 21 samples were fresh frozen (FF) and 63 samples were formaldehyde fixed paraffin embedded (FFPE) tissues (Table 1). The ovarian cancer tissue samples were comprised of serous, endometrioid, mucinous and clear cell subtypes of Stages I-III as determined by a pathologist. Using both FF and FFPE tissues allowed for determining potential differences in methylation between different histological tissue manipulations. It was determined by experimentation that methylation of the identified CpG dinucleotides was consistent between the FF or FFPE samples, as such tissue manipulation is not considered a variable when determining the methylation status of the identified CpG dinucleotides for ovarian cancer.

To identify differentially methylated CpG dinucleotides in ovarian cancer tissues, the methylation levels of CpG dinucleotides from the cancer tissues were compared to levels from normal ovarian and female reproductive tissue reference samples. Normal ovarian reference samples included 12 normal fallopian tube epithelial samples, 4 samples representing pooled normal ovarian surface epithelial cells and 2 normal ovarian tissues samples (Table 1). It was determined during experimentation that the methylation status of CpG dinucleotides was not significantly different among the normal samples, as such data from normal samples 1-18 were pooled and are reported as Normal Ovarian Tissue Type (Figure 3) and Normal (Table 2).

Table 1-Normal and ovarian cancer tissue sample characterization

Sample No.	Tissue Type	Tissue Characterization
1	Ovary_normal	Fallopian tube epithelium
2	Ovary_normal	Fallopian tube epithelium
3	Ovary_normal	Fallopian tube epithelium
4	Ovary_normal	Fallopian tube epithelium
5	Ovary_normal	Fallopian tube epithelium
6	Ovary_normal	Fallopian tube epithelium
7	Ovary_normal	Fallopian tube epithelium
8	Ovary_normal	Fallopian tube epithelium
9	Ovary_normal	Fallopian tube epithelium
10	Ovary_normal	Fallopian tube epithelium
11	Ovary_normal	Fallopian tube epithelium
12	Ovary_normal	Fallopian tube epithelium
13	Ovary_normal	Ovarian surface epithelial cells, pool 1
14	Ovary_normal	Ovarian surface epithelial cells, pool 2
15	Ovary_normal	Ovarian surface epithelial cells, pool 3
16	Ovary_normal	Ovarian surface epithelial cells, pool 4
17	Ovary_normal	Normal ovarian tissue
18	Ovary_normal	Normal ovarian tissue
19	Ovary_tumor FF	Stage **I** High-Grade serous
20	Ovary_tumor FF	Stage **I** High-Grade serous
21	Ovary_tumor FF	Stage **I** High-Grade serous
22	Ovary_tumor FF	Stage **I** clear cell
23	Ovary_tumor FF	Stage **I** endometrioid
24	Ovary_tumor FF	Stage **I** endometrioid
25	Ovary_tumor FF	Stage **I** clear cell
26	Ovary_tumor FF	Stage **II** High-Grade serous

27	Ovary_tumor FF	Stage II High-Grade serous
28	Ovary_tumor FF	Stage II High-Grade serous
29	Ovary_tumor FF	Stage II High-Grade serous
30	Ovary_tumor FF	Stage I mucinous
31	Ovary_tumor FF	Stage I mucinous
32	Ovary_tumor FF	Stage II High-Grade serous
33	Ovary_tumor FF	Stage II High-Grade serous
34	Ovary_tumor FF	Stage II High-Grade serous
35	Ovary_tumor FF	Stage I High-Grade serous
36	Ovary_tumor FF	Stage I High-Grade serous
37	Ovary_tumor FF	Stage I High-Grade serous
38	Ovary_tumor FF	Stage III High-Grade serous
39	Ovary_tumor FF	Stage II Low-Grade serous
40	Ovary_tumor_FFPE	Stage I endometrioid
41	Ovary_tumor_FFPE	Stage I endometrioid
42	Ovary_tumor_FFPE	Stage II High-Grade serous
43	Ovary_tumor_FFPE	N/A
44	Ovary_tumor_FFPE	Serous
45	Ovary_tumor_FFPE	Serous
46	Ovary_tumor_FFPE	Serous
47	Ovary_tumor_FFPE	N/A
48	Ovary_tumor_FFPE	N/A
49	Ovary_tumor_FFPE	Clear cell
50	Ovary_tumor_FFPE	N/A
51	Ovary_tumor_FFPE	Serous
52	Ovary_tumor_FFPE	Endometrioid
53	Ovary_tumor_FFPE	Mucinous
54	Ovary_tumor_FFPE	Endometrioid
55	Ovary_tumor_FFPE	Serous
56	Ovary_tumor_FFPE	Endometrioid
57	Ovary_tumor_FFPE	Mucinous
58	Ovary_tumor_FFPE	Serous
59	Ovary_tumor_FFPE	Serous
60	Ovary_tumor_FFPE	Clear cell
61	Ovary_tumor_FFPE	N/A
62	Ovary_tumor_FFPE	Endometrioid
63	Ovary_tumor_FFPE	Mucinous
64	Ovary_tumor_FFPE	Endometrioid
65	Ovary_tumor_FFPE	Serous
66	Ovary_tumor_FFPE	Serous
67	Ovary_tumor_FFPE	Endometrioid
68	Ovary_tumor_FFPE	Endometrioid

69	Ovary_tumor_FFPE	Clear cell
70	Ovary_tumor_FFPE	Endometrioid
71	Ovary_tumor_FFPE	Serous
72	Ovary_tumor_FFPE	Endometrioid
73	Ovary_tumor_FFPE	Endometrioid
74	Ovary_tumor_FFPE	Endometrioid
75	Ovary_tumor_FFPE	Mucinous
76	Ovary_tumor_FFPE	Mucinous
77	Ovary_tumor_FFPE	Serous
78	Ovary_tumor_FFPE	Mucinous
79	Ovary_tumor_FFPE	Serous
80	Ovary_tumor_FFPE	Serous
81	Ovary_tumor_FFPE	Endometrioid
82	Ovary_tumor_FFPE	Endometrioid
83	Ovary_tumor_FFPE	Serous
84	Ovary_tumor_FFPE	Serous
85	Ovary_tumor_FFPE	Serous
86	Ovary_tumor_FFPE	Serous
87	Ovary_tumor_FFPE	Clear cell
88	Ovary_tumor_FFPE	Endometrioid
89	Ovary_tumor_FFPE	Clear cell
90	Ovary_tumor_FFPE	Endometrioid
91	Ovary_tumor_FFPE	Endometrioid
92	Ovary_tumor_FFPE	Serous
93	Ovary_tumor_FFPE	Serous
94	Ovary_tumor_FFPE	Endometrioid
95	Ovary_tumor_FFPE	Serous
96	Ovary_tumor_FFPE	Endometrioid
97	Ovary_tumor_FFPE	Endometrioid
98	Ovary_tumor_FFPE	Serous
99	Ovary_tumor_FFPE	N/A
100	Ovary_tumor_FFPE	Serous
101	Ovary_tumor_FFPE	Serous
102	Ovary_tumor_FFPE	Mucinous
103	Ovary_tumor_FFPE	Endometrioid
104	Ovary_tumor_FFPE	Mucinous
105	Ovary_tumor_FFPE	Mucinous
106	Ovary_tumor_FFPE	Endometrioid

Additionally, 38 non-ovarian non-tumor tissue samples were included as general, reference samples, those tissues included adipose, adrenal, bladder, blood,

brain, breast, diaphragm, duodenum, heart, kidney, liver, lung, lymphnode, pancreas, skeletomuscle, skin, spleen, stomach, testis and ureter. It was determined during experimentation that the methylation levels of CpG dinucleotides from these disparate tissues did not align with those identified as being indicative of ovarian cancer, and indeed were akin to the levels found in the normal reference samples for the listed CpG dinucleotides (see Figure 3 Other Tissue).

The tissue samples were assayed for differential methylation utilizing the Infinium Assay for Methylation (I or II, one probe or two probes, respectively) followed by data analysis using GenomeStudio® Methylation Module vl.8 (Illumina, Inc.). Accuracy of a diagnostic assay or test can be measured by the area under a receiver-operator characteristic curve (ROC). The accuracy of a diagnostic test depends on how well the test can separate the group being tested, in this case ovarian cancer tissue samples, from those without the disease (normal ovarian tissue samples). An area of 1.00 represents a perfect test, 0.90-1 and excellent test, 0.80-0.90 a good, 0.70-0.80 a fair test and 0.50 and below a worthless test. Figure 1 shows an exemplary ROC curve for the first 100 methylation markers SEQ ID NO: 1-100. The ROC is very near 1.0 as such the use of the 100 markers is an excellent diagnostic test for ovarian cancer. Importantly, the samples' classifications that are used in computing the ROC are from samples not used in the selection of these markers. One way to summarize the ROC of Figure 1 is to calculate the area under the curve (AUC). Figure 2 represents AUC calculations for n=10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 biomarkers from Table 2. Data analysis identified 300 differentially methylated CpG dinucleotides with an area under the curve (AUC) of >0.992 as demonstrated in Figure 2. Additional biomarkers from the originally identified set of approximately 12,930 markers provided no real advantage in increasing the AUC and thus increasing the diagnostic ability of the proposed biomarkers. Further evaluation of CpG dinucleotide subsets of those 300 biomarkers was undertaken. It was determined that as few as 10 CpG dinucleotides, those represented by SEQ ID NO: 1-10 would yield an AUC of >0.992, or an excellent diagnostic assay for ovarian cancer. Further, p-values associated with each of the 300 methylation biomarkers disclosed herein demonstrate that each individual biomarker individually has utility as a diagnostic marker for ovarian cancer (Table 2).

As such, each of the 300 biomarkers disclosed herein, alone or in combination, finds utility in diagnostic assays for ovarian cancer.

Table 2 identifies 300 biomarkers useful in diagnostic and/or prognostic assays for ovarian cancer. The 300 biomarkers comprise those of known and unknown gene regions and adjacent regulatory regions. For identification purposes only, a methylation marker associated with a gene has been given the gene name as the Target ID to identify that particular marker, for example SEQ ID NO: 1 was given the Target ID of FOXM1 as this CpG dinucleotide is located in the gene region for forkhead box M1 (FOXM1). Those markers not correlated with a known gene region were given a Target ID starting with "eg", which is the naming convention generated by Illumina GenomeStudio software, for example SEQ ID NO: 2 Target ID is cg24871371 (as generated by Infinium (INF) I or II assay).

The beta-average (β-AVE) for both FF and FFPE ovarian cancer samples is reported as is the β-AVE for normal tissue samples. The β-average is the methylation level of the CpG dinucleotide in the group of samples; in this case the FF group, the FFPE group and the normal reference group (Table 1). The methylation status (METH STATUS) of the CpG dinucleotide biomarker (SEQ ID NOs: 1-300) identifies whether the methylation pattern of that particular marker in ovarian cancer tissue is increased relative to the normal reference control. For example, methylation of the CpG dinucleotide of SEQ ID NO: 1 is typically decreased relative to that of the normal control based on the β-average between the sample groups. Moreover, Table 2 reports probability values (P-VALUE) for each marker indicating how likely the observed difference would arise by chance. P-values were computed using the Wilcoxon rank sum test in R (for example, see www.r-project.com).

Table 2-Methylation biomarkers for ovarian cancer

TARGET ID	SEQ ID NO	INF	NORMAL β-AVE	FF β-AVE	FF P-VALUE	FFPE β-AVE	FFPE P-VALUE	METH STATUS
FOXM1	1	I	0.6239846	0.3627121	4.03E-15	0.4272913	7.86E-13	DEC
cg24871371	2	I	0.7361194	0.5078409	5.61E-13	0.5304895	2.54E-09	DEC
ZNF154	3	I	0.0630884	0.6749066	3.69E-12	0.4888479	1.49E-09	INC
ZNF154	4	I	0.0717513	0.6837412	4.89E-12	0.5421761	5.14E-08	INC
ZNF154	5	II	0.1450995	0.7302125	4.89E-12	0.6044022	8.42E-12	INC
GUCA1A	6	I	0.0895829	0.6767681	3.75E-11	0.4483576	7.11E-10	INC

ZNF154	7	11	0.2082342	0.7526685	2.76E-12	0.5854758	8.78E-08	INC
cg20935165	8	1	0.0920020	0.6419501	2.96E-11	0.4732697	1.55E-08	INC
ZNF154	9	11	0.2064662	0.7368401	1.50E-12	0.5651043	2.05E-07	INC
cg26579578	10	1	0.8863256	0.3683211	2.32E-11	0.5159614	1.33E-03	DEC
ZNF154	11	1	0.0667513	0.6042574	4.73E-11	0.4444550	3.91E-13	INC
ZNF154	12	11	0.2448174	0.7692188	3.69E-12	0.5958675	6.45E-07	INC
cg06255006	13	1	0.8664843	0.3211306	4.89E-12	0.5197226	2.11E-08	DEC
IMMP2L	14	11	0.8570674	0.3748570	4.89E-12	0.5639570	3.02E-09	DEC
PCDHGA4	15	1	0.1429221	0.6159599	2.96E-11	0.4159043	2.64E-06	INC
GPR25	16	1	0.1828292	0.7201829	4.89E-12	0.5324645	3.91E-07	INC
cg26869412	17	1	0.8862741	0.4103032	1.09E-11	0.5537760	1.19E-07	DEC
CDH4	18	1	0.8348529	0.3583556	5.94E-11	0.5670373	5.01E-05	DEC
cgl2021814	19	11	0.2722237	0.7398583	3.75E-11	0.5770035	5.70E-07	INC
ZNF154	20	11	0.2766132	0.7368445	1.41E-11	0.5677469	4.12E-06	INC
cg25259564	21	1	0.9150703	0.4038472	4.89E-12	0.5289431	5.94E-11	DEC
EMX20S	22	11	0.2282641	0.6276772	4.25E-09	0.5765402	3.30E-06	INC
cgl3924715	23	1	0.3831481	0.8940793	2.05E-12	0.8565577	2.32E-11	INC
cgl9125370	24	1	0.2350983	0.6919870	2.32E-11	0.6228840	1.49E-09	INC
cg24945701	25	1	0.2542991	0.6655342	6.44E-12	0.5318531	3.03E-07	INC
EMX20S	26	11	0.2937410	0.7584917	1.33E-08	0.6745536	1.18E-05	INC
PCDHB19P	27	1	0.3108483	0.7476680	3.75E-11	0.6285569	6.45E-07	INC
ZNF517	28	11	0.7418979	0.3309647	7.66E-14	0.3860852	8.07E-15	DEC
IRX1	29	1	0.2858995	0.7551908	1.09E-11	0.6558658	9.07E-08	INC
cgl6415411	30	1	0.7761870	0.3874091	7.86E-13	0.5036563	1.50E-12	DEC
MOSPD3	31	1	0.8728279	0.5032595	3.91E-13	0.6234320	3.02E-09	DEC
CDKN2A	32	11	0.1514849	0.6069571	4.73E-11	0.4628200	4.73E-11	INC
IRX2	33	11	0.3544379	0.7387569	1.82E-11	0.6747590	8.42E-12	INC
PRAME	34	1	0.5209022	0.1115715	4.84E-14	0.1544829	9.28E-11	DEC
cgl7278072	35	1	0.3332166	0.6974403	1.82E-11	0.6622541	3.59E-09	INC
PCDHGA4	36	1	0.2391787	0.6596428	3.75E-11	0.5148222	4.59E-06	INC
CADPS	37	11	0.3005552	0.7084067	3.01E-12	0.5389388	2.02E-05	INC
PCDHGA4	38	11	0.3493013	0.7071779	1.82E-11	0.5328378	1.81E-08	INC
JPH4	39	1	0.3162632	0.5865893	7.11E-10	0.5349135	4.80E-04	INC
cgl5092219	40	11	0.3265441	0.7030458	2.32E-11	0.5774651	4.57E-05	INC
CACNA1H	41	11	0.7213712	0.3561688	2.96E-11	0.4689980	2.05E-07	DEC
MELK	42	1	0.8479427	0.4568954	2.82E-14	0.5331823	7.86E-13	DEC
PCDHA6	43	11	0.3459174	0.6856542	1.09E-11	0.4667326	7.52E-04	INC
cgl0679156	44	11	0.3931305	0.7280242	3.75E-11	0.6258457	5.14E-08	INC
PCDHGA4	45	11	0.3636682	0.7061602	4.73E-11	0.5959607	1.33E-06	INC
PARP14	46	1	0.3932673	0.0783673	6.44E-12	0.0794765	1.21E-13	DEC
cgl2453631	47	11	0.3406015	0.6744751	1.82E-11	0.6049206	2.54E-09	INC
cg24394856	48	11	0.2809833	0.6494028	3.59E-09	0.5547573	1.18E-06	INC
PCDHGA4	49	1	0.2711145	0.6304705	4.73E-11	0.5678202	3.84E-08	INC
SLC25A2	50	11	0.3814967	0.7191509	2.96E-11	0.6046890	2.05E-07	INC
ZC3H4	51	11	0.6094863	0.1871093	8.07E-15	0.2610038	1.03E-09	DEC
cg25260543	52	11	0.2867451	0.6630305	3.75E-11	0.5296576	6.33E-06	INC
PRRT1	53	11	0.3432752	0.6940453	2.05E-12	0.5823320	2.54E-09	INC
cgl6625119	54	11	0.7109369	0.3833901	4.73E-11	0.5373494	3.14E-05	DEC
C2orf60	55	11	0.8190016	0.4415574	2.82E-14	0.5715456	3.02E-09	DEC
cg25153726	56	1	0.8724312	0.5354653	1.50E-12	0.6605657	2.11E-08	DEC
CDH4	57	11	0.7126432	0.3852444	7.44E-11	0.4827998	9.61E-06	DEC
CACNA1H	58	11	0.6373050	0.3687568	2.32E-11	0.4685589	4.57E-05	DEC

cg00257455	59	II	0.2090422	0.5431773	3.75E-11	0.3707384	2.14E-05	INC
EMX20S	60	II	0.4382136	0.7447125	3.26E-10	0.6678723	5.70E-07	INC
PCDHB11	61	II	0.3973011	0.7295614	2.96E-11	0.6229777	2.33E-07	INC
IRX2	62	I	0.3664338	0.7520240	6.44E-12	0.6355928	1.55E-08	INC
PTPRN2	63	I	0.8513516	0.5052185	1.41E-11	0.6160612	7.81E-06	DEC
cg09099868	64	II	0.3932816	0.7475700	3.75E-11	0.6192931	9.67E-09	INC
CLCC1	65	II	0.8481308	0.5123810	1.61E-14	0.5669782	7.11E-10	DEC
SFT2D2	66	II	0.4538990	0.2041238	8.23E-09	0.2347797	3.69E-12	DEC
cg04654288	67	II	0.2708411	0.6185688	2.32E-11	0.5910928	1.76E-10	INC
NRN1	68	II	0.3859314	0.7214555	5.61E-13	0.6213391	1.78E-09	INC
NKX2-8	69	I	0.2617161	0.5735972	5.94E-11	0.5042718	1.21E-04	INC
PCDHGA4	70	II	0.4870809	0.7901831	2.96E-11	0.7282945	3.26E-10	INC
PCDHGA4	71	II	0.3219265	0.6251675	3.75E-11	0.5350237	4.45E-08	INC
GUCA1A	72	II	0.4361103	0.7249411	2.05E-12	0.6562377	8.23E-09	INC
MY07A	73	II	0.5631352	0.2934881	1.10E-12	0.3914639	4.80E-04	DEC
NKX6-2	74	II	0.3344825	0.6315236	3.75E-11	0.5565776	5.94E-09	INC
cg20677570	75	I	0.4424854	0.6682544	1.82E-11	0.5996245	3.98E-10	INC
cg02324227	76	II	0.3968526	0.6853180	6.44E-12	0.5895194	5.03E-09	INC
PCDHA6	77	II	0.3154480	0.5875832	1.82E-11	0.5184445	1.24E-09	INC
DMRTA2	78	II	0.2709233	0.6105025	2.82E-14	0.5612537	4.44E-07	INC
PCDHGA4	79	II	0.5361474	0.7715370	3.75E-11	0.6605706	9.67E-09	INC
cgl5293759	80	I	0.9256887	0.6670976	4.89E-12	0.7500786	1.33E-08	DEC
CNTN5	81	I	0.8261678	0.5899548	2.32E-11	0.6093361	2.46E-08	DEC
PCDHB2	82	II	0.5587979	0.7902300	2.32E-11	0.6957271	4.45E-08	INC
cg04675342	83	I	0.8578001	0.5984908	2.32E-11	0.7177277	2.96E-06	DEC
PCDHGB1	84	II	0.3007868	0.5722716	8.42E-12	0.5006485	1.78E-09	INC
PCDHGA4	85	II	0.4908906	0.7620056	1.82E-11	0.6700410	2.86E-08	INC
PCDHGA4	86	II	0.4131446	0.6812413	1.41E-11	0.5673028	4.59E-06	INC
PCDHGA4	87	II	0.4793001	0.7168691	8.42E-12	0.6608540	2.11E-08	INC
MGC13005	88	I	0.6222524	0.3983102	4.84E-14	0.4463183	5.61E-13	DEC
PCDHB2	89	II	0.4120040	0.6388808	2.32E-11	0.5418681	3.44E-07	INC
cg00864171	90	II	0.3529857	0.6366827	7.66E-14	0.4803436	2.53E-02	INC
cgl0243855	91	II	0.6758184	0.3683456	8.07E-15	0.4790182	1.03E-09	DEC
FBX07	92	I	0.5806207	0.3439900	2.32E-11	0.3424016	2.05E-12	DEC
TUBB	93	II	0.3783210	0.1657484	8.07E-15	0.2282495	4.45E-08	DEC
KLHL8	94	II	0.5881427	0.2437406	2.82E-14	0.3052304	2.05E-07	DEC
PTPRS	95	II	0.7196312	0.4708134	3.91E-13	0.4701087	1.61E-14	DEC
cgl5208832	96	II	0.8576405	0.5382820	1.41E-11	0.6365453	4.45E-08	DEC
ALX4	97	II	0.4330429	0.7113429	1.41E-11	0.6384029	4.89E-03	INC
MCCC2	98	I	0.9003360	0.7008153	1.41E-11	0.7744557	5.03E-07	DEC
MELK	99	II	0.8302816	0.5140056	1.61E-14	0.5570156	2.05E-12	DEC
PCDHB19P	100	II	0.4361611	0.6795803	2.05E-12	0.5942857	2.54E-09	INC
F0XD4L1	101	II	0.3071273	0.5350926	7.86E-13	0.4508041	8.24E-07	INC
PCDHB10	102	II	0.3472116	0.5528921	2.32E-11	0.4837464	4.44E-07	INC
NKAPL	103	I	0.3151734	0.7562656	7.50E-11	0.6531139	1.63E-08	INC
GSTP1	104	II	0.7587147	0.3737301	1.61E-14	0.4276704	1.81E-13	DEC
cg00177787	105	I	0.8463402	0.3311613	7.00E-09	0.5003858	1.44E-04	DEC
cg25324047	106	I	0.1953967	0.7529362	3.91E-13	0.6257053	1.49E-06	INC
ZNF154	107	II	0.1725065	0.6900185	8.42E-12	0.5737264	3.26E-10	INC
LPAR5	108	1	0.2510296	0.6901494	4.73E-11	0.4358484	3.79E-05	INC
ZNF154	109	1	0.2304988	0.7243454	5.94E-11	0.5615655	2.54E-09	INC
MDC1	110	1	0.7509673	0.2876766	7.66E-14	0.3685954	4.03E-15	DEC

EMX20S	111	11	0.4008999	0.7601495	1.55E-08	0.6845225	4.16E-05	INC
EMX20S	112	11	0.3490223	0.7133246	1.33E-08	0.6188094	3.01E-04	INC
MMP23A	113	1	0.0793934	0.5560857	1.15E-10	0.3957134	5.94E-11	INC
ZIC4	114	1	0.1723636	0.6734545	3.69E-12	0.6041452	3.02E-09	INC
POLR2D	115	1	0.7836314	0.2718976	2.82E-14	0.4452172	2.66E-07	DEC
cgl6306259	116	11	0.8013733	0.3816228	6.85E-08	0.5475996	2.14E-05	DEC
ZNF154	117	11	0.2237296	0.7091964	6.44E-12	0.5931276	2.96E-11	INC
ALX3	118	1	0.2023519	0.6068597	3.03E-07	0.5089427	4.60E-03	INC
cgl7518215	119	1	0.2915967	0.7244579	6.44E-12	0.6006371	2.64E-06	INC
L1TD1;L1TD1	120	1	0.1969600	0.6201168	9.28E-11	0.3587767	5.59E-04	INC
PCDHGA4	121	1	0.1495023	0.5868392	5.03E-09	0.4789194	2.64E-06	INC
EMX20S	122	11	0.2918932	0.6620842	1.55E-08	0.5736245	2.18E-04	INC
PCDHGA4	123	1	0.1820442	0.6335130	3.59E-09	0.5189597	4.25E-09	INC
Cg06447424	124	11	0.2726524	0.7129886	4.84E-10	0.6204973	2.46E-08	INC
PRAME	125	11	0.8146420	0.3928016	7.88E-08	0.4874651	2.11E-06	DEC
ZNF572	126	11	0.8028735	0.3599886	2.54E-09	0.5348051	1.87E-03	DEC
TBPL2	127	1	0.2554441	0.6863139	4.73E-11	0.5273430	2.33E-07	INC
NKAPL	128	1	0.2191699	0.6337789	1.49E-09	0.4713663	1.59E-05	INC
cg24932585	129	1	0.2963044	0.7353868	2.05E-07	0.6317618	6.45E-07	INC
PIGB	130	1	0.9192126	0.4360791	2.82E-14	0.5238539	1.10E-12	DEC
cgl8813601	131	11	0.9033867	0.4848857	1.78E-09	0.5709761	1.82E-11	DEC
MMP23B	132	11	0.2387298	0.6744842	5.94E-11	0.5359084	4.44E-07	INC
TUBB	133	11	0.7768438	0.3685106	2.32E-11	0.4631253	1.43E-10	DEC
PCDHGA4	134	11	0.3785473	0.7359637	5.94E-09	0.6006008	6.85E-08	INC
cg08668316	135	1	0.2136801	0.6016228	7.11E-10	0.4847536	2.17E-10	INC
NFKBIL2	136	1	0.6523424	0.2485812	5.61E-13	0.3331853	3.26E-10	DEC
CD8A	137	1	0.4031034	0.7713802	7.44E-11	0.6697702	4.12E-06	INC
cg04727521	138	1	0.8194598	0.3669256	1.03E-09	0.5000322	7.03E-06	DEC
cg06708215	139	1	0.8827050	0.5088964	1.81E-08	0.5602422	5.94E-11	DEC
HLX	140	11	0.3184252	0.6691076	1.82E-11	0.5393048	5.01E-05	INC
cgl7555825	141	1	0.2151465	0.6307685	3.26E-10	0.5035489	5.14E-08	INC
cgll762968	142	11	0.3246807	0.7045310	1.82E-11	0.5552551	4.84E-10	INC
cgll305991	143	1	0.9085650	0.4386703	8.42E-12	0.5984299	2.19E-11	DEC
PTPRN2	144	1	0.7578228	0.3492184	1.03E-09	0.4074728	4.73E-11	DEC
cg02576528	145	1	0.8975170	0.5143567	7.88E-08	0.6530904	4.11E-04	DEC
EMX20S	146	11	0.3900872	0.7715855	3.84E-08	0.6762467	8.09E-04	INC
GRXCR2	147	11	0.8544075	0.4932695	8.23E-09	0.5601126	5.03E-07	DEC
cg06609496	148	1	0.7715672	0.3206744	1.81E-13	0.4654057	5.01E-05	DEC
VRK1	149	11	0.8649348	0.4899576	1.33E-08	0.5919623	1.49E-09	DEC
cg27228712	150	11	0.7904332	0.3987875	1.55E-08	0.4604942	4.84E-10	DEC
EMX20S	151	11	0.3033839	0.7008732	1.49E-09	0.5646200	1.56E-04	INC
TBPL2	152	11	0.3421740	0.7224708	1.15E-10	0.5675784	7.52E-04	INC
CDH4	153	1	0.8578149	0.4475274	5.94E-09	0.6032876	2.44E-03	DEC
PCDHGA4	154	11	0.2496987	0.6156388	3.02E-09	0.4749230	4.12E-06	INC
SLFN12L	155	11	0.2994400	0.7182721	6.44E-12	0.5725698	5.14E-08	INC
NKX6-2	156	1	0.2357596	0.6787889	2.96E-11	0.5863062	3.84E-08	INC
cg22550229	157	11	0.5226255	0.1871181	1.61E-14	0.2336168	1.50E-12	DEC
C1QL4	158	11	0.2894218	0.6584363	2.54E-09	0.5384561	2.01E-04	INC
cg21214521	159	1	0.7972023	0.3869529	3.32E-08	0.4896365	4.44E-07	DEC
cg25595388	160	1	0.6412315	0.2532649	7.88E-08	0.3864104	4.44E-07	DEC
TBX15	161	11	0.2765165	0.7145289	2.70E-13	0.6117190	8.23E-09	INC
S0X1	162	1	0.1095955	0.4796748	5.94E-11	0.4569508	5.87E-10	INC

SULT1A1	163	II	0.8599283	0.4676159	1.33E-08	0.5893953	2.11E-08	DEC
PCDHGA4	164	II	0.3403049	0.7109099	3.02E-09	0.6008763	2.54E-09	INC
MMP23B	165	II	0.2699230	0.6818197	2.32E-11	0.5458313	6.85E-08	INC
PRAME	166	I	0.5319860	0.1954143	2.66E-10	0.2301614	7.88E-08	DEC
NKAPL	167	II	0.3138315	0.6728251	1.24E-09	0.5359382	4.16E-05	INC
CDH4	168	II	0.7883417	0.4181665	3.75E-11	0.5219118	9.07E-08	DEC
NKAIN3	169	I	0.9521807	0.5570009	1.82E-11	0.7747810	1.57E-07	DEC
cg22677715	170	I	0.2243315	0.6195998	2.76E-12	0.4832623	1.78E-09	INC
C2orf60	171	II	0.7775256	0.4087839	1.33E-08	0.5164723	7.00E-09	DEC
PCDHGA4	172	II	0.3976157	0.7592808	1.24E-09	0.6491340	1.79E-07	INC
LOC494141	173	II	0.3304836	0.6883865	1.43E-10	0.5522495	2.86E-08	INC
PCDHGA4	174	II	0.3183327	0.6961383	2.32E-11	0.5503937	1.55E-08	INC
KCNT1	175	I	0.7184838	0.3501252	2.96E-11	0.3999622	6.44E-12	DEC
cg01713272	176	II	0.3309653	0.6723797	1.55E-08	0.6100177	1.57E-07	INC
cg00682734	177	II	0.3046838	0.6543142	7.11E-10	0.5549123	6.85E-08	INC
AQP11	178	I	0.6449935	0.2703838	1.76E-10	0.4365673	1.88E-06	DEC
MYT1L	179	II	0.8413884	0.4425031	9.67E-09	0.5092398	1.82E-11	DEC
CNST	180	II	0.5611915	0.1948099	1.10E-12	0.3158851	4.12E-06	DEC
C1QTNF4	181	I	0.2339239	0.7430809	8.42E-12	0.6036794	1.68E-06	INC
cgll687036	182	II	0.3174645	0.7040787	2.17E-10	0.6301908	1.78E-09	INC
DDX21	183	II	0.6579618	0.2878126	1.61E-14	0.3589343	5.94E-11	DEC
cgl3208088	184	II	0.4013217	0.7730892	1.09E-11	0.6671734	2.86E-08	INC
Clorf96	185	II	0.7339525	0.3779484	3.91E-13	0.5082763	3.91E-07	DEC
ZNF274	186	II	0.4166431	0.7913793	2.96E-11	0.6884680	1.81E-08	INC
cg22466850	187	1	0.8631796	0.5024547	1.10E-12	0.5655002	4.45E-08	DEC
C1QTNF8	188	1	0.8563137	0.4961259	7.66E-14	0.5922920	1.06E-05	DEC
cg00061811	189	1	0.8179498	0.4511518	3.91E-13	0.4977434	1.82E-11	DEC
EMX20S	190	II	0.1824731	0.5946469	1.24E-09	0.4639922	5.03E-07	INC
SRCIN1	191	II	0.4126121	0.7456657	1.09E-11	0.6614461	2.13E-09	INC
cg01891252	192	II	0.1598064	0.5510669	1.09E-11	0.4614731	1.81E-13	INC
IRX2	193	I	0.2204445	0.5706320	9.67E-09	0.4851822	1.05E-06	INC
cgll729934	194	II	0.3533979	0.6805124	6.85E-08	0.5984138	9.61E-06	INC
TAL1;TAL1	195	I	0.4184402	0.7427071	1.19E-10	0.6296756	2.47E-05	INC
cg02683197	196	II	0.3044763	0.6670738	9.28E-11	0.5244678	4.12E-06	INC
CDH4	197	II	0.7671742	0.3590006	1.41E-11	0.4394494	1.41E-11	DEC
cg07276621	198	II	0.4996864	0.8610773	7.86E-13	0.7790400	1.55E-08	INC
cgl6142855	199	II	0.3206157	0.7056020	1.43E-10	0.5818462	1.57E-07	INC
CACNA1H	200	I	0.8861176	0.4568655	4.89E-12	0.5934874	5.70E-07	DEC
cg05571581	201	I	0.0680891	0.4243915	3.26E-10	0.3214477	1.55E-08	INC
KNDC1	202	II	0.8576178	0.5388156	1.15E-10	0.6681649	3.84E-08	DEC
MGC2752	203	II	0.2688361	0.6393653	2.54E-09	0.5579693	2.46E-08	INC
MDC1	204	II	0.6032803	0.2854201	2.86E-08	0.4172412	3.82E-03	DEC
ZNF274	205	I	0.2613907	0.6444170	1.81E-08	0.6347750	1.78E-09	INC
ZNF274	206	II	0.4562188	0.8132808	5.94E-11	0.7508073	1.33E-08	INC
NRXN2	207	II	0.3592421	0.7026267	1.09E-11	0.5365198	3.91E-07	INC
cgll616547	208	II	0.2694195	0.6599373	7.44E-11	0.5171855	2.96E-06	INC
TBPL2	209	I	0.4468094	0.7479326	2.05E-07	0.6837291	4.16E-05	INC
cg07805777	210	I	0.3820992	0.7447308	4.73E-11	0.6453505	7.00E-09	INC
PCDHGA4	211	II	0.4374116	0.7723676	2.96E-11	0.6371523	9.61E-06	INC
MYT1L	212	II	0.7069598	0.3628823	3.91E-13	0.4518166	7.29E-07	DEC
cgl9539224	213	1	0.9553842	0.6320463	2.32E-11	0.7455151	2.32E-11	DEC
CBLN1	214	II	0.3560410	0.7050537	7.86E-13	0.5399653	1.75E-03	INC

cgl4506667	215	..	0.6655891	0.3247839	4.89E-12	0.3766237	1.81E-13	DEC
cg00812839	216	1	0.7230731	0.3418645	1.78E-09	0.4408748	5.03E-07	DEC
cg05740045	217	..	0.3734991	0.7410012	5.94E-11	0.6697884	3.32E-08	INC
cg26276327	218	..	0.3941211	0.7278125	2.46E-08	0.6072091	5.69E-06	INC
cg27322846	219	..	0.2362951	0.5983236	2.70E-13	0.4859855	6.44E-12	INC
KCNS1	220	|	0.2590614	0.6018991	7.11E-10	0.5177461	6.57E-05	INC
cgl3327545	221	|	0.2950498	0.6539814	3.75E-11	0.5815929	1.43E-10	INC
PCDHGA4	222	..	0.3745037	0.6699279	1.43E-10	0.5997774	2.17E-10	INC
CDH4	223	|	0.8387435	0.4495015	1.03E-09	0.5551217	1.61E-14	DEC
FAM188B	224	..	0.8025317	0.4173133	5.87E-10	0.4618098	3.26E-10	DEC
cg00589791	225	|	0.5357459	0.2336563	9.28E-11	0.2977399	8.23E-09	DEC
PCDHGA4	226	..	0.3103782	0.6267700	1.78E-09	0.4409766	6.57E-05	INC
CYP26A1	227	..	0.3882631	0.7077733	6.85E-08	0.5984971	9.07E-08	INC
PCDHGA4	228	|	0.3295497	0.7033860	4.73E-11	0.6261607	7.00E-09	INC
BARX1	229	|	0.3217299	0.6170340	1.49E-09	0.5095072	2.60E-05	INC
PCDHGA4	230	..	0.3748515	0.6701061	5.94E-09	0.5499821	7.88E-08	INC
KPNA5	231	..	0.8473592	0.4851916	1.61E-14	0.5602800	5.94E-11	DEC
cg23057567	232	..	0.5546916	0.2592713	5.87E-10	0.3243607	4.44E-07	DEC
FADD	233	|	0.6016083	0.2758273	1.33E-08	0.3921888	2.23E-02	DEC
PCDHB18	234	..	0.3198186	0.6603045	2.96E-11	0.5438912	1.33E-06	INC
PCDHGA4	235	..	0.2529543	0.6178413	1.78E-09	0.5821173	4.45E-08	INC
TBX5	236	..	0.2587574	0.6463125	1.82E-11	0.5475309	8.24E-07	INC
cg05107535	237	..	0.4500970	0.7743069	2.96E-11	0.7337161	1.41E-11	INC
NKAPL	238	..	0.3606733	0.6706031	4.45E-08	0.5774869	6.85E-08	INC
TCL1B	239	|	0.7521406	0.3643718	2.05E-12	0.4573986	3.03E-07	DEC
PAX6	240	..	0.3256841	0.7013662	2.70E-13	0.5823671	5.87E-10	INC
WISP1	241	..	0.8938278	0.5776590	2.96E-11	0.7136048	5.87E-10	DEC
GRIK2	242	..	0.4005255	0.6803570	3.75E-11	0.6660870	1.49E-09	INC
PCDHGA4	243	..	0.4609068	0.7506811	8.42E-12	0.6097405	1.18E-06	INC
PCDHB19P	244	..	0.2893917	0.6349325	3.26E-10	0.4876654	9.29E-07	INC
ZNF321	245	..	0.8470072	0.5133980	3.32E-08	0.6939213	3.14E-05	DEC
cg26801037	246	..	0.6732234	0.3662748	1.82E-11	0.4578056	8.66E-06	DEC
VENTX	247	|	0.6127130	0.2644001	2.96E-11	0.2764755	1.82E-11	DEC
cgl9767622	248	..	0.3241645	0.6986402	2.96E-11	0.6252814	1.55E-08	INC
STIM2	249	..	0.1705609	0.6568892	4.84E-14	0.4683478	9.67E-09	INC
RCC2	250	..	0.7193691	0.3817640	2.05E-12	0.4787900	3.26E-10	DEC
GSTP1	251	..	0.8053839	0.4584858	2.82E-14	0.5484876	2.19E-11	DEC
APRT	252	1	0.6384589	0.3073191	3.98E-10	0.4252145	2.05E-07	DEC
ZIC4	253	1	0.2246341	0.6017028	6.44E-12	0.5980598	3.59E-09	INC
cgl6596250	254	1	0.7609501	0.4313448	3.59E-09	0.5179234	5.14E-08	DEC
PCDHGA4	255	..	0.2907478	0.6371825	8.42E-12	0.4945129	9.29E-07	INC
SLC25A33	256	..	0.7640794	0.4777146	2.76E-12	0.4977512	2.54E-09	DEC
PRKAG2	257	..	0.8240676	0.3661740	2.76E-12	0.5126225	8.58E-10	DEC
RYR1	258	|	0.9197058	0.5935898	5.87E-10	0.6772151	6.01E-05	DEC
PEX19	259	..	0.8799780	0.5291691	1.24E-09	0.6616905	1.33E-08	DEC
SULT1A1	260	..	0.8099502	0.5037983	6.85E-08	0.5928083	2.36E-06	DEC
cg00652908	261	..	0.3308228	0.6381819	1.43E-10	0.5236577	5.94E-08	INC
cg08111158	262	..	0.4103389	0.7163784	2.96E-11	0.6000314	4.45E-08	INC
PCDHGA4	263	..	0.3541839	0.6520432	3.26E-10	0.5106944	1.82E-05	INC
GPR158	264	1	0.8272747	0.4198916	1.82E-11	0.6441701	1.85E-04	DEC
PTPRN2	265	1	0.8971776	0.5803257	1.33E-08	0.7015173	3.91E-07	DEC
ELFN1	266	1	0.7695046	0.3838100	1.81E-13	0.2335202	4.03E-15	DEC

ZIC1	267	1	0.1770567	0.5339313	8.58E-10	0.4753209	8.24E-07	INC
cg25318976	268	..	0.6586124	0.3413330	9.78E-12	0.3809873	5.92E-07	DEC
PCDHGA4	269	..	0.3541559	0.6697071	2.54E-09	0.5404440	2.36E-05	INC
TBPL2	270	..	0.3480699	0.6321779	5.87E-10	0.5180040	2.96E-06	INC
DKK3	271		0.6403540	0.3272544	7.11E-10	0.4458159	7.85E-03	DEC
RELB	272	..	0.6121272	0.3145991	1.13E-08	0.3716898	3.63E-06	DEC
cgl8143296	273	..	0.2983741	0.6444735	3.98E-10	0.5400797	2.64E-06	INC
CDH4	274	..	0.8322698	0.4714746	4.03E-15	0.5406054	1.15E-10	DEC
PCDHGA2	275	..	0.2449188	0.5548426	1.03E-09	0.4539590	2.66E-10	INC
cg07502874	276		0.8069306	0.4797621	7.44E-11	0.5761751	3.59E-03	DEC
KCND3	277		0.5990702	0.3270038	2.66E-10	0.4714442	8.31E-03	DEC
NLGN2	278	..	0.3434166	0.6842707	2.76E-12	0.5922213	2.96E-11	INC
KCNG3	279	..	0.2772322	0.6060475	3.32E-08	0.4738007	2.18E-04	INC
HELLS	280	..	0.8899214	0.5568681	5.94E-11	0.6749254	9.07E-08	DEC
CBX2	281		0.4183865	0.1224155	2.82E-14	0.2595035	1.87E-03	DEC
cg01164574	282	..	0.7427761	0.4027218	1.10E-12	0.4597286	1.81E-08	DEC
cg27582563	283	..	0.8254905	0.5000811	2.66E-10	0.5248240	1.15E-10	DEC
CACNA1A	284	..	0.7392866	0.4391084	7.88E-08	0.5285171	3.14E-05	DEC
SFT2D2	285	..	0.4972632	0.1511639	3.69E-12	0.1837706	7.11E-10	DEC
SLC26A5	286	..	0.5504728	0.2796902	1.50E-12	0.3450860	1.18E-06	DEC
cg04334235	287	..	0.8076243	0.5065123	4.45E-08	0.5605596	1.79E-07	DEC
cg09504320	288		0.3870570	0.7153616	4.73E-11	0.6665048	1.19E-07	INC
ELFN1	289		0.6182167	0.2535507	1.78E-09	0.3072492	1.33E-06	DEC
cg09757595	290	..	0.7796453	0.4338669	5.14E-08	0.5038159	2.36E-06	DEC
CREB5	291	..	0.5579471	0.7700913	4.73E-11	0.6629906	2.97E-03	INC
cg09552983	292		0.7637488	0.5328824	7.44E-11	0.5852852	1.85E-04	DEC
cgl7077610	293	..	0.3739958	0.7079120	1.21E-13	0.6315653	6.44E-12	INC
cg03061916	294	..	0.4044377	0.6844368	2.76E-12	0.5379621	4.44E-07	INC
NKX2-3	295	..	0.3554970	0.6925459	2.82E-14	0.6003929	3.91E-07	INC
cg22340508	296		0.3775989	0.6991871	5.87E-10	0.6092109	2.64E-06	INC
RPL30	297	..	0.8599504	0.5554443	1.41E-11	0.6151941	1.03E-09	DEC
cgl0640072	298	..	0.5163319	0.7500828	1.82E-11	0.6373960	8.66E-06	INC
cgl4015765	299	..	0.5139878	0.2010005	1.10E-12	0.2776742	5.03E-07	DEC
cg02610327	300	1	0.8435093	0.5743019	2.54E-09	0.6024864	1.10E-12	DEC

Figure 3 shows exemplary box plot graphs of subsets of methylation biomarkers and their advantage for determining the presence or absence of ovarian cancer. Methylation biomarkers SEQ ID NOs: 1-10 (10 Markers), SEQ ID NOs: 1-50 (50 Markers), SEQ ID NOs: 1-100 (100 Markers), SEQ ID NOs: 1-200 (200 Markers) and SEQ ID NOs: 1-300 are exemplified.

Table 3 reports the chromosomal location of the CpG dinucleotide methylation markers (CHR=chromosome, MAPINFO=location on chromosome) and the sequence (5' to 3' directionality) surrounding each biomarker CpG dinucleotide. Probes for detecting the biomarkers are easily developed by a skilled artisan for determining the

methylation status of one or more of the CpG dinucleotides as found in Table 3 for use in methods, for example as described herein.

Table 3-Location and sequence of methylation biomarkers for ovarian cancer

TARGET ID	SEQ ID NO	CHR	MAPINFO	SEQUENCE 5'-3'
FOXM1	1	12	2987138	GGGTGGATCACTTGAGGTCA[CG]AGTTCGAGACCAGCCTGGCA
cg24871371	2	6	161106477	GGTGCACCGTTTTTTAAGCC[CG]TCGGAAAAGCGCAGTATTCG
ZNF154	3	19	58220494	AGGGACGACGACTCCCCTCA[CG]CCTTCGTGGCCCCAACTCGG
ZNF154	4	19	58220370	GGGTAGAGCTGGGCCGGGAG[CG]ACGGGCGACATTGGTAGGGA
ZNF154	5	19	58220516	CCTTCGTGGCCCCAACTCGG[CG]CTCTGCTATCTCTGATCCGG
GUCA1A	6	6	42145954	GAGCCTTGGGTTATGATGGG[CG]GGGCCTGAGGCTGGAGTGAG
ZNF154	7	19	58220662	TTACTGGCTTAGTAGCGTGG[CG]TTCAACGCAGAGCATTCTAG
cg20935165	8	2	172972840	CTGTTTCCGGCCAGGGGACG[CG]AGAGAAGGCGGCACCACACT
ZNF154	9	19	58220657	AGCTCTTACTGGCTTAGTAG[CG]TGGCGTTCAACGCAGAGCAT
cg26579578	10	11	69707303	CGCGCCTGTCCGCGGTGGAG[CG]CACCTGTCCGCGGTGGAGCG
ZNF154	11	19	58220080	CCCTGCAGAAAACAGCTTTC[CG]AATTCTCCTGGCTCAGTCGC
ZNF154	12	19	58220718	ATAGATCCCGAGGTGGGTGC[CG]GGGACCCTTTGCACCAACCT
cg06255006	13	16	86653215	TTCCACGGAAATGACTCCGA[CG]ACCCAGATGTTACTACCTTT
IMMP2L	14	7	111203008	AGTGAGTAACACAGGACAGG[CG]GTTTACTTGAGAGAAACGGG
PCDHGA4	15	5	140800929	GAGACCGAAAAGGGCTCCTT[CG]TGGGCAATATCTCCAAGGAC
GPR25	16	1	200842890	GGGTCGGGCCCGGAGGAACT[CG]CTGCGCATCATCTTCGCCAT
cg26869412	17	10	3023801	TGCCGGGGTTGCTTACACCC[CG]CCGGAAGCTAGAGGATCAGG
CDH4	18	20	60470267	GGCTGCTGTGTGGAGTGGCT[CG]ATTCCTGATGTGCGCAGTAG
cg12021814	19	2	172973195	TAAACCCCAGGGGCTTGAAC[CG]AAATCATCGGTTACTCGACG
ZNF154	20	19	58220773	CGAAGCTCCAGGGCGCTGGG[CG]ATGAGAAATGGCTTATCCAA
cg25259564	21	11	1060659	CCAGGCCGCTGACATGGGGA[CG]CGCTAGAGCTTTCGAACACG
EMX2OS	22	10	119296297	CCTTGCTTTCAATAATGAAT[CG]AGACAGCGTGTGTGTGGTGA
cg13924715	23	11	10750890	CGCCCAAATCTCACACAGTG[CG]TCTGATTCGCTGAGCCTTAA
cg19125370	24	6	10390532	CCCCCCTTCTAGCCCAACTC[CG]CCAGCCTCCCCGCCCCCGCC
cg24945701	25	3	62362783	CAGCCCGTCACGCACGGCCGC[CG]GGCCCGAATCCACCTTGGCG
EMX2OS	26	10	119293083	AGCACCAGCTCAGTACAAGG[CG]CTGGAGGGGAAGGAGGGAAG
PCDHB19P	27	5	140621375	CTGACAGGCTGCTGAGCGAG[CG]TGACACAGCCAAGCACAGGC
ZNF517	28	8	146023573	CAAGATCCCAGAAACTGGCC[CG]GTGCGGTAGCTCACGCCTGT
IRX1	29	5	3599686	AAGGCCGAGGACGACGAGGAGATCGACCTGGAAAGCATCG
cg16415411	30	7	6910871	TTTTGATAGTCCAATTCATCCG[CG]CTCCACCTTTCCGGAATGCT
MOSPD3	31	7	100208950	ATCGCTTGGGCCCAGGAGTT[CG]AGAACAGCCTGCAGAACATA
CDKN2A	32	9	21968233	GCCTCTCTGGTTCTTTCAAT[CG]GGGATGTCTGCAGAGGGCAG
IRX2	33	5	2750163	CCATCTTCCCCTAAGTGTTC[CG]GAGCCGCTCGTGGCAATGTA
PRAME	34	22	22901830	TCCTGGTAAGGGGAGAGGGA[CG]GGCTAGGAGCCAAGCGGAAG
cg17278072	35	7	155258411	CGAGCTGCGCCCCAGGGTCAT[CG]CTGATGGCGCGAGACCACGC
PCDHGA4	36	5	140800761	CGATTCACAACCAACCAGCT[CG]AGAAACCGCGGAATATCGGC
CADPS	37	3	62861544	CTGATTCCAGCAACTTATTC[CG]CATGCGCCCAGTCTAATTAA
PCDHGA4	38	5	140801354	CTTGCAACTGCGGGGCAGAA[CG]GATGGGGCCAAGAATCCAGA
JPH4	39	14	24045274	CCGCTGGCCTCCGAGCCGGG[CG]GTCCGGTGCTGCCCACCTCGC
cg15092219	40	1	247511364	GAGCCCGGCGGGCTCTGGGG[CG]CTGAGCTCATCTCGCCACGC
CACNA1H	41	16	1271505	ACCACCCTTTCCGTTCCGCT[CG]GGCCTTCCCAGAAGCGTCCT
MELK	42	9	36572421	TTTAGCCAGGCGTGGTGGCG[CG]TGCCTGTAGTCTCAGCTACT
PCDHA6	43	5	140228182	AGCGGCCAGCTCCACTACTC[CG]TCCCGGAGGAAGCCGAACAC
cg10679156	44	6	27228101	TGCCCAGGACCATTTAGGGT[CG]CGGTGACCGGAGAACCACCC

PCDHGA4	45	5	140801482	GAAAGGGCGCAGTTCCCATT[CG]TGTGGTGGTCCTCGATGTAA
PARP14	46	3	122400474	GGCCACATTGGAGGGGCTGT[CG]ATCGACCACAGGTGCCCATT
cgl2453631	47	3	157812226	CG[illegible]GCCCGA[illegible]GA GCA[illegible]G[CG]AA [illegible]GGGGCCAAGAAA [illegible]G
cg24394856	48	6	134175900	AGCGTGCAGGGGATGGGCTG[CG]ACCGCTCCCAGTGACCCCAC
PCDHGA4	49	5	140802432	GACCCG CCCCTCAG CAGCAA [CG]TGTCG CTGA GCCTGTTCGTG
SLC25A2	50	5	140683367	GGCTGCCCAGTCAGTACACA[CG]CTGTCCCCCCTGCGGCCCCC
ZC3H4	51	19	47618017	TTCCAGGAAGAGAGAAAAAC[CG]TGTCAACAGTGATAAATCTG
cg25260543	52	7	100946792	GGCGGCTGAACACTCCTCCC[CG]GTGTCTGAGTTCAGGGGCCC
PRRT1	53	6	32118204	CTG CAG CTG CAG GGGGTATC [CG] GGCGCTACGTAGCCCCCCAG
cgl6625119	54	16	86653176	CAGTGTG CCCAGTCAGTGGG [CG] TG GGCGTTACCACTCCTTTC
C2orf60	55	2	200819113	AGGATTACTGGCATGAACCA[CG]GCGCCCAGCCCATCCGACTT
cg25153726	56	13	111217366	TAGCCCCTGACAATGACTAA[CG]GACTCATTTGACTCTCATCA
CDH4	57	20	60470081	TGACAGATGTGAATGACAACC[CG]CCAGAATTTACCGCCAGCAC
CACNA1H	58	16	1255116	CCCCACCTCTG CCTG CAGCC [CG] AGGAG CTG ACTAATG CTCTG
cg00257455	59	1	65530538	CTTTAATTCATATTCTGAAA[CG]TGCTTGTTATTGTTGACGTA
EMX20S	60	10	119296756	AAACCA AGGACCCCCTGTGT [CG] GGCGTCTTCAG CCAG CCG CG
PCDHB11	61	5	140580769	CTGCACATCGGCAGTGTCAG[CG]CTACAGACAGAGACTCAGGC
IRX2	62	5	2750758	GAAACCTGGGCGCCTCGGGC[CG]GACTCCGGAGCCGCAGCTGA
PTPRN2	63	7	157777033	GCTCCAGGCTCCACCCCATCCCA[CG]CCAGGCTCCACCCAATCCCA
cg09099868	64	10	22622793	CACCGTGGACGTGATTTCAA[CG]TTGGTGCGGATTTGGAGATG
CLCC1	65	1	109506696	A[illegible]AAAGGGAGGA [illegible]CA[CG] AA [illegible]GACAA[illegible]G [illegible]AG [illegible][illegible]AC
SFT2D2	66	1	168194826	AGTAG CTG GG CTTCCAGG CA [CG] CCCCACCACACCAGACTAA
cg04654288	67	6	10390247	TGTTAAATG CTCCCGTCGTT[CG] CAG GGG CTG GGACTTGATAA
NRN1	68	6	6000601	GACTAAATATAATCCCAACC [CG] TGATCGATTCCTGGGGCGCT
NKX2-8	69	14	37053169	GACCTGGCTCGGATGCGGCGG[CGJGCGGCCCTGTCAAGCCCCGC
PCDHGA4	70	5	140789745	GCCACTCTCTG CCACCG CCA [CG] CTTCATCTGGTCTTCGCAGA
PCDHGA4	71	5	140741730	GAGGTATTGCCAGACCTCAG[CG]ACCGCCGGGAGCCCTCTGAC
GUCA1A	72	6	42146143	GGCAACGGCTGCATTGACCG[CG]ATGAGCTGCTCACCATCATC
MY07A	73	11	76902992	[illegible]GGAC[illegible][illegible]CCC[illegible]CCCC[illegible][CG]C[illegible]GGGCC[illegible]CC[illegible][illegible][illegible][illegible]C
NKX6-2	74	10	134598496	GTTTGTGCTTCTTGAGCAGC[CG]CGTGATCTTCTCGTCGTCCG
cg20677570	75	11	15962841	GCTCTCCAG CAAGTCG CTCC [CG] GGTCCCAG GCCAAG GG CTG C
cg02324227	76	5	180591594	GGAATTCGAACCCATGCCTC[CG]AAGACACCGGCGCCTTAATC
PCDHA6	77	5	140214328	GCTGCAG GTTTTCCATGTGGA[CG]TGGAGGTGAAG GACATTAAC
DMRTA2	78	1	50887477	TTTGTCTGAATTAGTGATTC[CG]CTGGAAAAAATAAAGAGACA
PCDHGA4	79	5	140803130	CCAGCCCAACTATGGGGACA[CG]CTCATCAGCCAGGAGAGCTG
cgl5293759	80	10	86040024	CACCCGAATATTGCGCTTTT[CG]GACCAGCTTAAAAAACGGCG
CNTN5	81	11	100156776	ACC[illegible]AA [illegible]A[illegible]G CG[illegible][illegible][illegible]C[CG]A [illegible]A GG[illegible][illegible]AAAAAACGGCCC
PCDHB2	82	5	140475611	TGATAAGCACGGCCCTGGAC[CG]GGAGACCAGATCCGAATACA
cg04675342	83	10	113822450	CACCTGAATACTGCGCTTTT[CG]GACGGGCTTAAAAAACGGCG
PCDHGB1	84	5	140731930	ATCTCAGTGCTCTTTCTCCT[CG]CGGTGATTCTAGCGATCGCC
PCDHGA4	85	5	140794962	GGCGGCTTGACAGGTGTGTC[CG]GCTCGCACTTTGTGGGCGTG
PCDHGA4	86	5	140800983	CCCCGGGAGCTGGCGAAGCG[CG]GAGTCCGCATCGTCTCCAGA
PCDHGA4	87	5	140794664	AGCCCTGCTGGACAGAGACG[CG]CTCAAGCAAAGCCTCGTAGT
MGC13005	88	2	114361816	CACG GCAG GGCTCTCTTG CT[CG] CAGTATAGTG GTG GCATGCC
PCDHB2	89	5	140476324	GTGCTGGTCAAGGACAATGG[CG]AGCCTCCGCGCTCGGCCACC
cg00864171	90	11	67383662	GATGGGGCCCGGGCTCACAT[CG]ATGGCCATGTGCTCCTGCATG
cgl0243855	91	17	1096705	AGCACGTAACCTTCACTCTT [CG] CAAG CAG CCTCTCTATGTGG
FBX07	92	22	32870183	CAG GCTG AGGTG CAGTG GTG [CG] ATCACAG CTCATTGA GGCCT
TUBB	93	6	30687221	CACCTGGTCAGTGAAATCAG[CG]AATTGAAAAACCACTGACTT
KLHL8	94	4	88142277	GCAGTG CAAGTG GCCATGCG [CG] GTG GCTCACG CCTGTAATCC
PTPRS	95	19	5341595	CTG ACAACAG CTACCAGCCA [CG] CCCCACTCCACTCTATCCCA
cgl5208832	96	1	65467997	GCTCGGTACCGCCACGCACC[CG]TCCTATGTGTTACCTGGTCG

ALX4	97	11	44332958	CGCGTGCAGCCCCTCGATTT[CG]CTTCATGACAACCGTGCTAG
MCCC2	98	5	70906136	GG[illegible]CCGAAAAGCGCAA[illegible][CG]GG[illegible]GGA[illegible]GACCCGA[illegible]
MELK	99	9	36572411	CCACAAATAATTTAGCCAGG[CG]TGGTGGCGCGTGCCTGTAGT
PCDHB19P	100	5	140621344	AGGGCTGTTCGGCGTGTGGG[CG]CACAATGCACTGACAGGCTG
FOXD4L1	101	2	114257020	AGCCGGGGCTGCAGGTGGCC[CG]GTGGGGCGGGGTTGCGCTTC
PCDHB10	102	5	140574095	TCCTCGCTCGGCCACCGCCA[CG]CTGCACTTGCTCCTGGTGGA
NKAPL	103	6	28227127	GCCTCTGGGTCTGTAGCAAC[CG]CCCAGCGTTGAGGCGCGGCT
GSTP1	104	11	67350491	AACTTCAAATAAAAGTTGGA[CG]GCCAGGCGTGGTGGCTCACG
cg00177787	105	10	3330480	GCGTGAGGTGAGGGCTCCCC[CG]CGCCGCCCTGGCGTCCAGGT
cg25324047	106	10	22765840	ACGCTCCCCAATTTCCCCAT[CG]AGCCTTCTCCTCCCGAGTCT
ZNF154	107	19	58220295	GGTGTGTGAGGACGGGAAGA[CG]CCGCACTCACCTGAGTTGGC
LPAR5	108	12	6729718	GTTAGCCAGCAGGAGGCGCA[CG]GTCTTCCGCCGCCGCTGGCT
ZNF154	109	19	58220818	CTAGGGCAGTGGAGGGACTT[CG]CCCTTTCTTAAATGGGTCGT
MDC1	110	6	30684284	CAGCAGTGAGCAACCGCGCC[CG]GCCTCAAATGTCACTTTCTC
EMX20S	111	10	119295770	TGAGAGTGTGGCTCCTGGGA[CG]CCAGCGTTTCAGAAGTAGCA
EMX20S	112	10	119295875	CTCCAAGTTAAAAGGCCGGC[CG]GGCTATCAGTGGCAGAGAGC
MMP23A	113	1	1566699	CTGGACGCCGGCCGCCCCGT[CG]AACCTTTGGGTCTCCGAGCT
ZIC4	114	3	147108843	GAATCGTAGCCAGAGCTGGG[CG]GCGGCGAGCGCCCGTGCACC
P0LR2D	115	2	128616167	TTAAGCAAATGACGGCCGGG[CG]CAGTGGCTCACGCCTGTAAT
cgl6306259	116	6	1595099	GCCCTCACCATAACCGATGG[CG]ACAACTGAGGCAGGCCCAGG
ZNF154	117	19	58220837	TCGCCCTTTCTTAAATGGGT[CG]TAATCAGACAGCATATTAGA
ALX3	118	1	110610899	GAGCCGG[illegible]A[illegible]TT[illegible]AAAAAC[CG]GGAAA[illegible]AAGGCGGG[illegible]CCC[illegible]
cgl7518215	119	2	172973241	CAGCCGTGGCCTTTCCGCGG[CG]CTGGGCTTCTGGTGCTATCA
L1TD1;L1TD1	120	1	62660861	TGGTCCAGGCGCGAAGGGCG[CG]GGGTGCCCCGGGTAAGGCTG
PCDHGA4	121	5	140800586	AATCAGGGAATGGGAAGCTG[CG]CGCCATTGAGTCCCTCCCTC
EMX20S	122	10	119296182	TGCACCACCGTCTGTGTGTA[CG]TCTGTCTGTCCCTCCCAGTC
PCDHGA4	123	5	140810106	TAATTGGTTAGGACTCTGAG[CG]CCGCTGTTCACCAATCGGGG
Cg06447424	124	13	20875743	CTGGGTCTTGCCCTCTGGAA[CG]GCAGCAGGCGCGCTCCCGGC
PRAME	125	22	22901267	AGTCTCGCCCCACCCCGCCqCG]CAAGTCTAGAAAAGATGCCC
ZNF572	126	8	125984927	ATATATATCCATGTTCATAG[CG]GTGGGAACAGAAACAATAGG
TBPL2	127	14	55907299	CAGCGAGGCGGGGCGGCCCT[CG]GCCCAGGTTCCTGCAGAGGG
NKAPL	128	6	28227091	TTCTAGTGCGCCTGCGTGGC[CG]CGAATCACCAGCCAGCCTCT
cg24932585	129	19	12666317	GCTGGAGGGCGGGGATGTCC[CG]GGACAGGCCGCGGCCCCTGC
PIGB	130	15	55610624	[illegible]AACAAGAGGAG[illegible]GGGC[CG]CACGCGG[illegible]GGC[illegible]CACGC[illegible]G
cgl8813601	131	10	3330571	CGGAGCGGCTGCGTGCAGCC[CG]CAGGTGAGACCGTCTGCATA
MMP23B	132	1	1566351	GCAGGGAGTTAGTTGGGGGG[CGJTCCCAGGCAGGGTCTGGGGG
TUBB	133	6	30687197	CTCTTGCAGAGTTGCAGTGG[CG]GCCACCTGGTCAGTGAAATC
PCDHGA4	134	5	140810123	GAGCGCCGCTGTTCACCAAT[CG]GGGAGAGAAAAGCGGAGATC
cg08668316	135	4	380157	GGAGGCCTCATCAGAACCGA[CG]GGAAATGGCGGCGGCGGGAC
NFKBIL2	136	8	145670656	TCCGAGCGCTATGGGAGGCC[CG]GTCAGGAGGATCGCTTGAGC
CD8A	137	2	87036626	TGGGTGCCGGGGCCCCGAC[CG]GGGCTGAGCTGGTCCCCTGG
cg04727521	138	16	1154951	TGCTCCTCTCCGTGGCTGCA[CG]TCCTGGCCACCCCGAGGCCG
cg06708215	139	10	134648037	TGGGAGCAGGCAGGCGGCGC[CG]AGTCCTGAACAAAGGCGGCC
HLX	140	1	221054273	TGTGGCGTTCTTGGAAGACA[CG]TGAAAGTGAGGCCGTAAGCC
cgl7555825	141	5	76924190	CGCACGCAGCCCGCGAGGGG[CG]CCCTCCGCGCCACTGCCCCA
cgll762968	142	13	95354190	GAGGAAGGCCTTAAGGCCCA[CG]GAAGCCTCATCCCGCCAAGC
cgll305991	143	10	131844324	GTGGTAGGGCTCAGGGCAGA[CG]GAACGTGGAACAGGGAGTGG
PTPRN2	144	7	158045996	CGGGCGGGGGCCTGAGGACT[CG]AGGACTGACCGGGGCCAGCAC
cg02576528	145	10	131845025	GGCCACCAGCTCGCCCACGC[CG]CCCGCTCACCTGCACGCGGA
EMX20S	146	10	119295827	ACGCTGACAAACCCAAGTAG[CG]CCTTACATTGTCGGGCGAGG
GRXCR2	147	5	145252094	CCAACAGTTCTGGTTGGTAC[CG]GAGGGGGAGCAAGACACAAA
cg06609496	148	5	1174426	GCTCCAGGGCACCCCCTCCC[CG]GGATGCCCCACACGAAGCACCCG

VRK1	149	14	97262732	AAGTGAGGTCCTGAAGCTTA[CG]CTTCATTGGCTTCATTGTTA
cg27228712	150	7	570041	TTTCCTCCACACCCAGCGTC[CG]GATGCTCTGTGATGTGTTAA
EMX20S	151	10	119294055	TCCCTTCGAGCGCCCTGCAG[CG]GAGTGTTAGACAAGCCCTGT
TBPL2	152	14	55907198	CCCACTGTTGGGGGTGGGGG[CG]GGTAAGAGGGTAAGCGCGGA
CDH4	153	20	60470166	GGCAGGAATGCACTGGCGTG[CG]GCACAGCCTCCTCTCTCTCT
PCDHGA4	154	5	140810260	CAACTGTCCCATTCTATGGG[CG]AAGGAACTGCTCCTGACTTC
SLFN12L	155	17	33823690	GTGCAGTAGGCGTCTGTCTT[CG]CGGTGGCAGCA
NKX6-2	156	10	134598352	AATTTATTGATGATACAAAG[CG]ACTCGCGCCCACCCGGGGCC
cg22550229	157	18	13138034	CTGAGCAGGCTCAAGGGCTG[CG]TTATACAATCTTCTCGGTCC
C1QL4	158	12	49730055	CTGGTGGTCCAGGGGGCCCC[CG]CAGGCCTGCTTTCCCGCGCC
cg21214521	159	7	569979	GTCCTCAGGGCGCGACACGA[CG]CTGCCCGCTGAGGCGTCAGA
cg25595388	160	5	2684004	CCACCGTGAGTCTGGAAGCC[CG]TGGAGGGTGCCTGCAGCCCT
TBX15	161	1	119528638	GGCCCTAGACGGGCTCCGTG[CG]ATCTGGGGCCTCCCAAGAGA
SOX1	162	13	112723477	ACAAAAAAG CAAAACGAGG[CG]AGAGGCGAAGCCCAC TG
SULT1A1	163	16	28635374	GTCTCGAGTGATCTGCCCGC[CG]CGGCCTCCCATATGGGGTTA
PCDHGA4	164	5	140810161	GATCCTGCTCGCCTTGCACG[CG]CCTGAAGCACAAAGCAGATA
MMP23B	165	1	1566374	CCCAGGCAGGGTCTGGGGGC[CG]GGCGCACGCAGGCGGGGTGA
PRAME	166	22	22901857	GGAGCCAAGCGGAAGGACCC[CG]TGTTCAAGGCCCTTCAAGGG
NKAPL	167	6	28227093	CTAGTGCGCCTGCGTGGCCG[CG]AATCACCAGCCAGCCTCTGG
CDH4	168	20	60349210	CTGCCTTCTCTGCACAGAGC[CG]GCCCTCCCACCTCAGCACCC
NKAIN3	169	8	63662874	TATTTGAGTGCTTTGACCAC[CG]ACACATCTCCTCCATGAATA
cg22677715	170	2	162284644	CGAGCCCAGAGCAGCGGGGA[CG]GGCGTCCGGGAGCTCGCCCG
C2orf60	171	2	200819070	CCTGACCTCAAGTGATCCAC[CG]ACCTGGGCCTCCCAAAATGT
PCDHGA4	172	5	140810137	ACCAATCGGGGAGAGAAAAG[CG]GAGATCCTGCTCGCCTTGCA
LOC494141	173	11	18230903	GGAGGGAGCAGAGCTGGCAC[CG]CGCAAGGGCCCCTGCCTCCC
PCDHGA4	174	5	140801286	GCTAGGGATCCAGATGTGGG[CG]TGAACTCCCTCCAGAGCTAC
KCNT1	175	9	138662115	GCAGCCATGGCCGAGGGTGA[CG]CTCCCCTGGCCCCGCCCTGG
cg01713272	176	18	12911711	TTCTGGAGGGCGTCTGAGGT[CG]TCAGCAGCGGCCTACGACTT
cg00682734	177	20	55200973	CTGTGAGCCCCTCAGCTCCT[CG]CCTCACTTTGCCTGTTTGAA
AQP11	178	11	77299805	GAGCCACCGCGCCCAGCCG[CG]CCCGG
MYT1L	179	2	2019937	TGTTTGGGTGCAGGGACAA[CG]CTGTGGTGAAACCATACAAA
CNST	180	1	246728663	ATACTTTGTGGTGTCATTAA[CG]ATACAGACTCTACGCTTATG
C1QTNF4	181	11	47611780	GAGTGGTTGGTGCCGCGGCC[CG]GGGCCAGCGTCCGAGCCCAC
cgll687036	182	3	147139563	GCTCCCCAGTGAGGCGGGAG[CG]AAAAGAGTGCAGGCCCACTC
DDX21	183	10	70717302	TTTAGAATTGTAGGAAGCCG[CG]GCAGATTTGTACTCAGGTGC
cgl3208088	184	6	10392519	GCGCA CC[CG]GGCA AAAGA GAAAAAGA
Clorf96	185	1	229480105	ATAGCTAAATGATTCAGAGG[CG]GCTACTGGCCCAAATGGGGC
ZNF274	186	19	58715577	AAACTCCTGCCGCACTGCCC[CG]TTGCCCCATGTAGGCCCAGG
cg22466850	187	7	93220797	TGTCGGAAAAGCGCAGTATG[CG]GGTGGGAGTGACCCGATTTT
C1QTNF8	188	16	1146445	\GGCACGCAGCTCTCCTCCAT[CG]GGAAACCAAGCCTGCTGCCC
cg00061811	189	6	170475845	AGGCAGGTGGGCGGCCCTTC[CG]GGGGCGTGGCCAGGCAGGTG
EMX20S	190	10	119293800	AGCAGAGCAC[CG]AGAAGGGGAACC CAACA C
SRCIN1	191	17	36719654	ATGCCCATGGTGAGCTTCTG[CG]GGAACATGTGCGCGATGAGT
cg01891252	192	1	50881219	CCCAGAAGCGGAGACTCAAT[CG]CCCCCCTGCCCCTGCTACCA
IRX2	193	5	2751041	CAGCCGGCGAGCCGAGGGGA[CG]CGCGGGGGGCGCGCGGGTCC
cgll729934	194	6	1601693	CCAGCTCTCAGTGAGGACTT[CG]CGGTCCTGGGGCCAGTAGGT
TAL1;TAL1	195	1	47694919	GAATAGGATCTCCACTCCGC[CG]GAAAGGGGCGGAAGCCGAGG
cg02683197	196	6	28174875	GCAGTGCTCCAGTACTGGTT[CG]ATTTCCGCAAAGTCCTTCTT
CDH4	197	20	60472331	CCATTGCAAGCCCTACCCCT[CG]TCTCTGGTCCCCATCCCGAG
cg07276621	198	11	10750870	CAAGGGTCTTCGTTGGCTTC[CG]CCCAAATCTCACACAGTGCG
cgl6142855	199	4	379900	TGCCCAATCAGGGATGCAGC[CG]GAGAGGAGAGGGCGGCATCC
CACNA1H	200	16	1213894	GGGCTATGTGGCGGCCACCT[CG]AAGGCCAGGTCTGGGGACCC

cg05571581	201	6	28753940	TGCGGCTGCGGCAGCAAAGG[CG]GAGGAGGAGCGAAGTGGACG
KNDC1	202	10	135018992	AGGTTCCGGAGAATCACACC[CG]GGCGTGCACTCACATTTACA
MGC2752	203	19	59092692	GGAACACAAGCACCCCCTTG[CG]GTGTCTGGAGGAAGCGGCGC
MDC1	204	6	30684478	CAGGACTGGATTAGGGGAAC[CG]TGTCTTTCCCCTAGGGTCCA
ZNF274	205	19	58715677	CTGCTGGGAGCTGTAGGCCC[CG]CGGGTGTCGTAGTTCTGGGC
ZNF274	206	19	58716004	ACTAGTAATTTTCTGCTCTG[CG]ACCGTTTTTCTGCGCTGTAA
NRXN2	207	11	64398015	GGTAATGTCGTCCGTGCCCA[CG[TTAAAG ATCACCCCCACGGT
cgll616547	208	6	28782301	TCGCGGCGTTGAGAACGCCT[CG]CAGCTCCTTTACTGGCTGGG
TBPL2	209	14	55907427	AGGGGTGGGCCGGAGAGGGG [CGJTCTCGCCTGGGGGCAGTCAC
cg07805777	210	11	15962932	GCCTGGGGGGCCGGGCAGGC[CG]AGGCCCCTGCCCGTCGCAGT
PCDHGA4	211	5	140798095	GGTGGGGACCCTCCCCGAAG[CG]GTACTGCTCAGATAAG AATC
MYT1L	212	2	1926724	GTTTTCTGAGAGCACAACAC[CG] TGTCCTTGGGCTAATAGTTT
cgl9539224	213	3	125439871	CTTATGGATCGGCTCAGACAA[CG]GGCC TGCATTTTTGGCTGCC
CBLN1	214	16	49312543	GAAGAAGCTCCTTGGCGTTT[CG]TGGAGTCACAGAGCACGACG
cgl4506667	215	14	106410734	ACCGAGCCGGGGCTTTCCCA[CG]TGCCTCCTTACAATTGCTAT
cg00812839	216	1	146895247	ACCCGAATACTGCGCTTTTC[CG]ACCAGCTTAAAAAACGGCCC
cg05740045	217	8	65499926	GGGCGCAATTGTCCTTCAAC[CG]CCAAGCTCCTTGGGACAAAA
cg26276327	218	1	247511469	GGCTGGGCCGCAGCGGACGC[CG]GCTCCCCGATCACCCGCTCA
cg27322846	219	2	162284638	TGCACCCGAGCCCAGAGCAG[CG]GGGACGGGCGTCCGGGAGCT
KCNS1	220	20	43726544	TACTGGGCGCCAGCAGGAGG[CG]CGACGACACCTCGAAGCTGA
cgl3327545	221	10	22623548	CGCTCTCCAAAGTTGGACCC[CG]TGGCGAGCGGCGGCGACAGC
PCDHGA4	222	5	140810726	ACGACAATGCGCCTTACTTT[CG]TGAAAGTGAATTAGAAATAA
CDH4	223	20	60116795	CTTTTCTCTCACTTAATG[CG]TCTGTGAGCCTCTCCCCGTG
FAM188B	224	7	30810183	AAACCATCCAGCTGACACCT[CG]ATCTTGGTCTGTAGCCCTCA
cg00589791	225	5	30429329	AGTCAGAAAAGCGCATTATT[CG]GGTGGGAGTGACCTGATTTT
PCDHGA4	226	5	140800398	CTTTTAATAATTACTCCTTT[CG]CAGGTGTTCTTGGTAAAGTT
CYP26A1	227	10	94835255	AGCTGCGGAAGGGGCTGCGG[CG]GAACTGGGAGCATCCCCTAG
PCDHGA4	228	5	140810920	CCCGAATTGGTGCTGAAACG[CG]CCCTGGACCGCGAAGAAAAG
BARX1	229	9	96716209	GTCTCCAGGCTAGGGAGCAC[CG]ACTAGGAGGTGGGGGGGTGC
PCDHGA4	230	5	140810404	CTGGGGACTCTGTGGGAGAC[CG]GATGCACCCAGATACGCTAT
KPNA5	231	6	117001170	TATGGACTCATGATTGGCAA[CG]CTTTCTGGTAACACTGCCCA
cg23057567	232	19	47017629	TGTTTTCCATTTTAAAA TCCTTCG]TTTTTAGTTTGCAATTTCTC
FADD	233	11	70048796	CCACGACCTTCTCTCTTCTGGAG[CG] TGTTCGCTTTTTGTAATTAGA
PCDHB18	234	5	140616495	AGCCCCACCTTTCTGAATGG[CG]TGGAATGCAATTAGGGATCTG
PCDHGA4	235	5	140744367	ACGGTTAGTGCTTCCCTTCG[CG]CGGGATGCGGATGTGGGTGT
TBX5	236	12	114845868	AAGAGGCAACCAGGCGATAG[CG]ACTATCTCACCAGCCGCTGC
cg05107535	237	16	3242850	GCTTTGCAGTTTGAGCTTTTTGG[CG]GTCCGGTGGGGTGTCTGACTT
NKAPL	238	6	28227220	GAGAAGGCGACGCAGCTCCT[CG]GGGAGCCCACCATCCCCGCA
TCL1B	239	14	96152706	CCCCGCCCCCACTGCCGGCC[CG]GGCCCCACCCACGCCGGAGC
PAX6	240	11	31819219	GCAGCCAGGCGGTGACCTAG[CG]GCTGCTCTTACATAAAATGG
WISP1	241	8	134224890	CGCTGACCAGCGGAAGGATT[CG] GGCAATTGGTTTAACTTCGC
GRIK2	242	6	101846767	CAAAACCTTTTGCTAGCAAAG[CG]CC TTTTTGTGCCGGGCTGGG
PCDHGA4	243	5	140802135	CTGAATGTGCAGATGACAA[CG] ATAACCCTCCCGTTTTTCCT
PCDHB19P	244	5	140619446	CAGAGCAGATTGCCTACCAA[CG]CAACATAGGCATCCGGACCC
ZNF321	245	19	53446957	AGGAAAACTCTTAGAAGTTG[CG]TCTGCAGCCGGGCGCCGGTG
cg26801037	246	7	157294357	TCGCTCAGAGCCATTGTGCA[CG]TTTGTTTCTCTGATCACTAA
VENTX	247	10	135055156	CACCCCAACAGGAACAGAAG[CG]TGGTCCTGCGGCTGCGTCCC
cgl9767622	248	6	164051738	TAGCAGCAATAGAGAACTGA[CG]CCAGCGCCTGCCACGGTCCA
STIM2	249	4	26994740	GGCTGGGGATTTTGTGGG[CG]TGTGAAGGTACAGCTGCACCA
RCC2	250	1	17766917	CAGTGGAACGCACAGCCTAA[CG]GAAAGACAGATCAGTAAACA
GSTP1	251	11	67350499	ATAAAAGTTGGACGGCCAGG[CG]TGGTGGCTCACGCCTGTAAT
APRT	252	16	88879593	AGGGCGGGTGGGGGACCCAT[CG]TCTGATGCCAAGGGGCGTGG

ZIC4	253	3	147108916	TGTAGCACTTGTCGCAGCCC[CG]CACCTTGCACGTGTATGGCT
cgI6596250	254	7	53099739	GGGCCGTTTTTTAAGCTGGT[CG]GAAAAGCGCAA TATTTCGGGA
PCDHGA4	255	5	140802831	CTGGCGGACCTCGGCAGCCT[CG]AGTCTCTGGCTAACTCTGAA
SLC25A33	256	1	9600721	TATTTAATTTTGAGGACCTTCA T[CG]GCCTGGCACAGGAA TTTTTG
PRKAG2	257	7	151548036	GTCACTTGAGCTCCAAGGCA[CG]GCCCACAGGGGTACCCCTGC
RYR1	258	19	38974117	GGCCGTGCAGTGCCAGGAGC[CG]CTGACCATGATGGCGCTGCA
PEX19	259	1	160255758	TACCGTACTTTTTTATTCTC[CG]CTCCCACCCTGCACGAATTT
SULT1A1	260	16	28635371	CTGGTCTCGAGTGATCTGCC[CG]CCGCGGCCTCCCATATGGGG
cg00652908	261	16	46878164	AGTGGAGGCCGTGCGCACCG[CG]AGCTCAACACAGTTGGGGGC
cg08111158	262	1	227746191	GCTGCAGCCCGGGACCTCCT[CG]TGGGGGTCCACGATTGTAGC
PCDHGA4	263	5	140811253	ACAATAGGGGAGTTGGACCA[CG]AGGAGTCAGGATTCTACCAG
GPR158	264	10	25755241	CCGTGAGACCACGAACCCTT[CG]ATCGAGAAAAGACCTTCAAT
PTPRN2	265	7	158049906	GACCAGCGTGTGGAGAGACC[CG]GGCACACGGCTGCTCAGGAC
ELFN1	266	7	1773245	GGGCGGCCGCATCCCAGCCC[CG]TCCCAGCCCATCTTCCGTTG
ZIC1	267	3	147128123	GCCAGGCTACGCGGCTGCTG[CG]GCCCTGGGCCATCACCATCA
cg25318976	268	1	5727352	CTCTCTGGCTGAGCCTTGAT[CG]TGTTCAAGCCACAACCACAG
PCDHGA4	269	5	140800424	TGTTTCTTGGTAAAGTTTTAA[CG] TCACACTAA TGTAAG TATTTGT
TBPL2	270	14	55907460	GGCAGTCACCCCACTCTGCC[CG]CAGCTGGGAAGGGCCTGGGC
DKK3	271	11	12031266	CCTGGCATTCAAGCCATCAC[CG]CTGGCCTTAGTCTGCCGTGA
RELB	272	19	45504295	TTGAGGAAACTGAGGTGTAG[CG]AGACCTTGGAGGTTTCCGAA
cgI8143296	273	3	157812763	CAAGGCCTGCGCACTGAATA[CG]GCCCAAATCCTGTTCAGTGG
CDH4	274	20	60397766	GGGCTTCACTGAGTTTGGGG[CG]GGAAAAGG TCAA TTTTCA TC
PCDHGA2	275	5	140735027	CAGCGGCACCTTGGTCACCG[CG]GGTAGGATAGACAGGGAGGA
cg07502874	276	16	1040578	AGCTGGGCTTGGTGCGGCTG[CG]GCCAGTACGCTGGTCCTGCC
KCND3	277	1	112438746	GGCAGGTGTGACCAGCCCCA[CG]GTGCTGAGGGAAGCAGAGGC
NLGN2	278	17	7311620	TGGGGCTGGCGGGGGCTCAA[CG]CGGGGGAGGGGGTCCCGGCG
KCNG3	279	2	42720326	GCAGCAGTACTCGAGGTGCG[CG]CCCTCCAGGCCCCAGTAGAT
HELLS	280	10	96304193	ATGTCCGACACTAAAATGTC[CG]GGCGTGGTGGCACGCACCTG
CBX2	281	17	77751069	CGGACAATTGATCCGAAGGA[CG]CCGGCTGCCCAGCCCTGGCG
cg01164574	282	21	22368940	AAAGGGTGTGAGAAGGGTAC[CG]TGGCAAAATAACTTTCTAAA
cg27582563	283	2	20384285	TGCTGGGAACATCCACTTCC[CG]ATGCCTCCTCCTTTGACGTC
CACNA1A	284	19	13366101	AGAGAGGCCAGTGGTGAGAG[CG]GCAGAGGCAGGAGGAAGGTG
SFT2D2	285	1	168196658	TTGTGGTTGCTGATTTTATT[CG]GTTTTTGTTTTGTGATTACT
SLC26A5	286	7	103087458	GAGCACCGCAGCCAGAGTGC[CG]GGCTCCAAGAGGGTGAGGGG
cg04334235	287	14	106188793	TCCACCACCAGACAGGTGAT[CG]TGGGCGACTTGCGGATGAAC
cg09504320	288	1	157164796	CGCACTTGTGCACAGAGTAC[CG]GCAGGCACCAATCGCCCGGC
ELFN1	289	7	1782346	CAAGCCCTGGGTTAGCTCCC[CG]AGGCCCGCTAGGACCTCGTT
cg09757595	290	2	151504597	TTCTTGCTGAACCATTCTCAG[CG]TGTTTGAGTTACTTGAGGCA
CREB5	291	7	28448103	CTCGCTGCAGACCAGGCGCC[CG]GATCCTGCAGTCTGGCCCTG
cg09552983	292	3	178578041	CGAATACTGCGCTTTTCCGA[CG]GGCTTAAGAAACGGCGCACC
cgI7077610	293	10	22766143	AAGCATATGCTGCACCTCTG[CG]CCGGTTAAAATCACCCCCAG
cg03061916	294	10	94829090	CTCGAAAGGGCTCAAGGTCAC[CG]GATTCTGCTGGCCACTTCTT
NKX2-3	295	10	101294300	TTTACACTCAGAACAGA TTTTT[CG]CGCAACCCA TGCCCACCGGC
cg22340508	296	19	22891978	CCGGGGTCGCCTAGCCCAGGAA[CG]CCTTAGTTGCAACCCTGCGT
RPL30	297	8	99059026	AAGTTGATTCAGGCCGAATG[CG]GTGACTCACGCCTGTAATCC
cgI0640072	298	8	99985888	ATGAAGCCTCTCCACGTGGC[CG]ACTTCCCTTAGAGAAGTCCC
cgI4015765	299	10	132734310	GGCCTGAGGATGGAGGGCCA[CG]ACTTCTGTGTGCTCAAGAGC
cg02610327	300	4	44169592	GCTGGTCTCCAGCTCCTGAC[CG]CGAGTGATCTGCCTGCCTGG

The methods and biomarkers described herein can be applied to the characterization, diagnosis or prognosis of ovarian cancer characterized by a pattern of one or more methylated genomic CpG dinucleotide sequences that is/are distinct from the pattern of one or more methylated genomic CpG dinucleotide sequences exhibited in the absence of ovarian cancer. Pattern of methylation as used herein refers to whether a particular biomarker (from a test sample) demonstrates an increased or decreased level of methylation relative to the level of methylation of that biomarker in a reference sample. Methylation of CpG dinucleotide sequences can be measured using any of a variety of techniques used in the art for the analysis of specific CpG dinucleotide methylation status. For example, methylation can be measured by employing a restriction enzyme based technology, which utilizes methylation sensitive restriction endonucleases for the differentiation between methylated and unmethylated cytosines. Restriction enzyme based technologies include, for example, restriction digest with methylation-sensitive restriction enzymes followed by Southern blot analysis, use of methylation-specific enzymes and PCR, restriction landmark genomic scanning (RLGS) and differential methylation hybridization (DMH).

Restriction enzymes characteristically hydrolyze DNA at and/or upon recognition of specific sequences or recognition motifs that are typically between 4- to 8- bases in length. Among such enzymes, methylation sensitive restriction enzymes are distinguished by the fact that they either cleave, or fail to cleave DNA according to the cytosine methylation state present in the recognition motif, in particular, of the CpG sequences. In methods employing such methylation sensitive restriction enzymes, the digested DNA fragments can be separated, for example, by gel electrophoresis, on the basis of size, and the methylation status of the sequence is thereby deduced, based on the presence or absence of particular fragments. Preferably, a post-digest PCR amplification step is added wherein a set of two oligonucleotide primers, one on each side of the methylation sensitive restriction site, is used to amplify the digested genomic DNA. PCR products are not detectable where digestion of the subtended methylation sensitive restriction enzyme site occurs. Techniques for restriction enzyme based analysis of genomic methylation are well known in the art and include the following: differential methylation hybridization (DMH) (Huang et al, 1999, Human Mol. Genet. 8, 459-70); Not I-based differential

methylation hybridization (for example, WO02/086163A1); restriction landmark genomic scanning (RLGS) (Plass et al, 1999, Genomics 58:254-62); methylation sensitive arbitrarily primed PCR (AP-PCR) (Gonzalgo et al, 1997, Cancer Res. 57: 594-599); methylated CpG island amplification (MCA) (Toyota et. al, 1999, Cancer Res. 59: 2307-2312). Other useful methods for detecting genomic methylation are described, for example, in US Patent Application publication 2003/0170684 or WO 04/05122.

Methylation of CpG dinucleotide sequences can also be measured by employing cytosine conversion based technologies, which rely on methylation status-dependent chemical modification of CpG sequences within isolated genomic DNA, or fragments thereof, followed by DNA sequence analysis. Chemical reagents that are able to distinguish between methylated and non-methylated CpG dinucleotide sequences include hydrazine, which cleaves the nucleic acid, and bisulfite treatment. Bisulfite treatment followed by alkaline hydrolysis specifically converts non-methylated cytosine to uracil, leaving 5-methylcytosine unmodified as described by Olek A., 1996, Nucleic Acids Res. 24:5064-6 or Frommer et al., 1992, Proc. Natl. Acad. Sci. USA 89:1827-1831. The bisulfite-treated DNA can subsequently be analyzed by conventional molecular techniques, such as PCR amplification, sequencing, and detection comprising oligonucleotide hybridization.

Techniques for the analysis of bisulfite treated DNA can employ methylation-sensitive primers for the analysis of CpG methylation status with isolated genomic DNA as described by Herman et al, 1996, Proc. Natl. Acad. Sci. USA 93:9821-9826, and in U.S. Patents. 5,786,146 and 6,265,171. Methylation sensitive PCR (MSP) allows for the detection of a specific methylated CpG position within, for example, the regulatory region of a gene. The DNA of interest is treated such that methylated and non-methylated cytosines are differentially modified, for example, by bisulfite treatment, in a manner discernable by their hybridization behavior. PCR primers specific to each of the methylated and non-methylated states of the DNA are used in PCR amplification. Products of the amplification reaction are then detected, allowing for the deduction of the methylation status of the CpG position within the genomic DNA. Other methods for the analysis of bisulfite treated DNA include methylation-sensitive single nucleotide primer extension (Ms-SNuPE) (Gonzalgo & Jones, 1997;

Nucleic Acids Res. 25:2529-2531, and see U.S. Patent 6,251,594), and the use of real time PCR based methods, such as the art-recognized fluorescence-based real-time PCR technique MethyLight™ (Eads et al, 1999; Cancer Res. 59:2302-2306, U.S. Patent 6,33 1,393 and Heid et al, 1996, Genome Res. 6:986-994). It is understood that a variety of methylation assay methods can be used for the determination of the methylation status of particular genomic CpG positions. Methods which require bisulfite conversion include, for example, bisulfite sequencing, methylation-specific PCR, methylation-sensitive single nucleotide primer extension (Ms-SnuPE), MALDI mass spectrometry and methylation-specific oligonucleotide arrays are described, for example, in U.S. Patent 7,61 1,869 and International Patent Application WO2004/051224.

In one embodiment, methylation of genomic CpG positions in a sample can be detected using an array of probes. In particular embodiments, a plurality of different probe molecules can be attached to a substrate or otherwise spatially distinguished in an array. Exemplary arrays that can be used in the invention include, without limitation, slide arrays, silicon wafer arrays, liquid arrays, bead-based arrays and others known in the art or set forth in further detail herein. In preferred embodiments, the methods of the invention can be practiced with array technology that combines a miniaturized array platform, a high level of assay multiplexing, and scalable automation for sample handling and data processing.

An array of arrays, also referred to as a composite array, having a plurality of individual arrays that is configured to allow processing of multiple samples can be used. Exemplary composite arrays that can be used in the invention are described in U.S. Patent 6,429,027 and US Patent Application publication 2002/0102578 and include, for example, one component system in which each array is located in a well of a multi-well plate or two component systems in which a first component has several separate arrays configured to be dipped simultaneously into the wells of a second component. A substrate of a composite array can include a plurality of individual array locations, each having a plurality of probes and each physically separated from other assay locations on the same substrate such that a fluid contacting one array location is prevented from contacting another array location. Each array location can have a plurality of different probe molecules that are directly attached to

the substrate or that are attached to the substrate via rigid particles in wells (also referred to herein as beads in wells).

In a particular embodiment, an array substrate can be a fiber optical bundle or array of bundles, such as those generally described in U.S. Patents 6,023,540, 6,200,737 and 6,327,410; and PCT publications WO9840726, W099 18434 and WO9850782. An optical fiber bundle or array of bundles can have probes attached directly to the fibers or via beads. Other substrates having probes attached to a substrate via beads are described, for example, in US Patent Application publication 2002/0102578 and US Patent 6,770,441. A substrate, such as a fiber or silicon chip, can be modified to form discrete sites or wells such that only a single bead is associated with the site or well. For example, when the substrate is a fiber optic bundle, wells can be made in a terminal or distal end of individual fibers by etching, with respect to the cladding, such that small wells or depressions are formed at one end of the fibers. Beads can be non-covalently associated in wells of a substrate or, if desired, wells can be chemically functionalized for covalent binding of beads. Other discrete sites can also be used for attachment of particles including, for example, patterns of adhesive or covalent linkers. Thus, an array substrate can have an array of particles each attached to a patterned surface.

In a particular embodiment, a surface of a substrate can include physical alterations to attach probes or produce array locations. For example, the surface of a substrate can be modified to contain chemically modified sites that are useful for attaching, either-covalently or non-covalently, probe molecules or particles having attached probe molecules. Chemically modified sites can include, but are not limited to the linkers and reactive groups set forth above. Alternatively, polymeric probes can be attached by sequential addition of monomeric units to synthesize the polymeric probes *in situ*. Probes can be attached using any of a variety of methods known in the art including, but not limited to, an ink-jet printing method as described, for example, in US Patents 5,981,733; 6,001,309; 6,221,653; 6,232,072 or 6,458,583; a spotting technique such as one described in US Patent 6,1 10,426; a photolithographic synthesis method such as one described in US Patents 6,379,895 or 5,856,101; or printing method utilizing a mask as described in US Patent 6,667,394. Accordingly,

arrays described in the aforementioned references can be used in a method described herein.

The size of an array used in the invention can vary depending on the probe composition and desired use of the array. Arrays containing from about 2 different probes to many millions can be made. Generally, an array can have from two to as many as a billion or more probes per square centimeter. Very high density arrays are useful in methods described herein, for example, those having from about 10,000,000 probes/cm^2 to about 2,000,000,000 probes/cm^2 or from about 100,000,000 probes/cm^2 to about 1,000,000,000 probes/cm^2. High density arrays can also be used including, for example, those in the range from about 100,000 probes/cm^2 to about 10,000,000 probes/cm^2 or about 1,000,000 probes/cm^2 to about 5,000,000 probes/cm^2. Moderate density arrays useful in methods described herein can range from about 10,000 probes/cm^2 to about 100,000 probes/cm^2, or from about 20,000 probes/cm^2 to about 50,000 probes/cm^2. Low density arrays are generally less than 10,000 probes/cm^2 with from about 1,000 probes/cm^2 to about 5,000 probes/cm^2 being useful in particular embodiments. Very low density arrays having less than 1,000 probes/cm^2, from about 10 probes/cm^2 to about 1000 probes/cm^2, or from about 100 probes/cm^2 to about 500 probes/cm^2 are also useful in some applications.

Methods for determining ovarian cancer described herein can provide a robust and ultra high-throughput technology for simultaneously measuring methylation at many specific sites in a genome. The methods further provide cost-effective methylation profiling of thousands of samples in a reproducible, well-controlled system. In particular the disclosed methods allow implementation of a process, including sample preparation, bisulfite treatment, genotyping-based assay and PCR amplification that can be carried out on a robotic platform.

The methods can be carried out at a level of multiplexing that is 96-plex or even higher including, for example, as high as 1,500-plex. An advantage of methods described herein is that the amount of genomic DNA used for detection of methylated sequences is low including, for example, less that 1 ng of genomic DNA per locus. In one embodiment, the throughput of the methods can be 96 samples per run, with 1,000 to 1,500 methylation assays per sample (144,000 data points or more per run). In the embodiment exemplified herein, the system is capable of carrying out as many

as 10 runs per day or more. A further object of the disclosed methods is to provide assays to survey methylation status the 5'- regulatory regions of at least 1,000 human genes per sample. Particular genes of interest are tumor suppressor genes or other cancer-related genes, as well as genes identified through RNA profiling.

In preferred embodiments, methods for assaying samples for the presence of ovarian cancer based on methylation status of one or more CpG dinucleotides comprises a bead based assay. Genomic DNA is initially quantitated by any known method. However, DNA quantitation methods such a PicoGreen (Molecular Probes) are preferred as this method can quantitate small amounts of DNA and measures DNA directly, thereby minimizing detection of contaminating RNA and/or proteins. The present method is not limited to any particular method for DNA quantitation.

Once quantitated, an amount of the genomic DNA can be processed and analyzed for methylation status. A genomic DNA sample is exposed to sodium bisulfite for conversion of unmethylated cytosines to uracils, while leaving methylated cytosine unchanged for methylation analysis. Methylation detection in bisulfite converted DNA is based on the different sensitivity of cytosine and 5-methylcytosine to deamination by bisulfite. Under acidic conditions, cytosine undergoes conversion to uracil, while methylated cytosine remains unreactive. Bisulfite conversion of DNA is well known in the art and there are numerous commercial kits and methods, for example EZ DNA Methylation Kit by Zymo, for practicing bisulfite conversion of DNA for methylation analysis. The present method is not limited to any particular method for bisulfite conversion of DNA, however complete conversion of cytosine to uracil is highly recommended as incomplete conversion can lead to false positive methylation signals, and thusly reduce the quality and effectiveness of a diagnostic assay.

Once genomic DNA has been bisulfite converted, the sample can be denatured, for example by addition of NaOH, and the resultant single stranded DNA neutralized for in preparation for amplification. Single stranded DNA can be isothermally amplified such that whole genome amplification occurs resulting in a uniform increase in the amount of DNA sample and minimizing amplification bias which can occurs during thermal (e.g., traditional polymerase chain reaction at high temperatures). Isothermal amplification can be carried out, for example, by

incubating the DNA sample overnight at 37°C in the presence of dNTPs, DNA polymerase with strand displacement activity and primers for example as described in US Patent publication 2008/0009420 (incorporated herein by reference in its entirety). The present method is not limited by the type of amplification performed for increasing the amount of DNA for analysis; however an unbiased method for amplification is preferable.

Following isothermal amplification, the amplified, bisulfite converted DNA can be fragmented. Fragmentation can be by any means, however enzymatic or chemical fragmentation is preferred as it is more easily manipulated to provide a more homogenous mixture of fragments. For example, the sample can be incubated in the presence of a cleavage enzyme or chemical at 37°C for a period of time (e.g., 1 hour, etc.) to generate DNA fragments, for example of between 100-lOOObp long. The fragments of DNA can then be precipitated out by any means, for example by using 2-propanol according to standard techniques. The precipitated fragments can be washed and resuspended in a resuspension buffer, for example a Tris-EDTA based buffer, for subsequent hybridization to a substrate for methylation analysis.

Following fragment precipitation, the fragments can be hybridized to a substrate for analysis. For example, beads comprising immobilized (e.g., covalently bound) probes for capturing genomic DNA can be incubated with the DNA fragments such that hybridization of the fragments to homologous probes immobilized on the beads is carried out. The fragmented DNA, under hybridization conditions known to a skilled artisan, can by allowed to hybridize to the bead probes, for example overnight at 48°C, thereby immobilizing the fragmented DNA for methylation assay. The beads can be in a tube or deposited on a substrate such as a slide, for example a slide that allows for multiple bead populations and multiple samples to be assayed at one time, or multiplexed applications. Such applications are found in the Illumina's INFINIUM beadchip Methylation Assays using one probe (I) or two probes (II) affixed to beads which are located on slides such that one probe can be used to interrogate a CpG dinucleotide sequence or two probes can be used to interrogate a CpG dinucleotide sequence, depending on the CpG location and how resulting probe design. The substrate containing the immobilized, fragmented DNA can be washed to remove unbound DNA fragments.

Following removal of unbound DNA fragments, the probes bound to the fragmented DNA can be extended, for example by single base extension, by providing dNTPs, DNA polymerase, and other components necessary for DNA extension to occur. The single base extension can incorporate one or more labeled nucleotides, for example hapten labeled nucleotides such as biotin and nitrophenol (DNP) labeled nucleotides. For example, to differentiate between the four dNTPs, adenines and thymines could be biotin labeled and cytosine and guanines could be DNP labelled. Following single base extension, fluorescently labeled binding partners to the labeled nucleotides are introduced and fluorescence of the extensions is detected, for example by a fluorescent scanner. Software can then be used to decipher the fluorescence data captured by the fluorescent scanner and methylation status along with additional data parameters can be output to the user. For example, GENOMESTUDIO Methylation software can be used to analyze methylation data from scanned microarray images collected from fluorescent scanners and output a variety of reports, graphs, tables etc. characterizing the sample(s) methylation status.

Therefore, embodiments described herein make available diagnostic and/or prognostic assays for the analysis of the methylation status of CpG dinucleotide sequence positions as markers for ovarian cancer. The methods described herein provide a systematic method for the identification, assessment and validation of genomic targets as well as a systematic means for the identification and verification of multiple condition relevant CpG positions to be used alone, or in combination with other CpG positions, for example, as a panel or array of markers, that form the basis of a clinically relevant diagnostic or prognostic assay. The disclosed method enables differentiation between two or more phenotypically distinct classes of biological matter and allows for the comparative analysis of the methylation patterns of CpG dinucleotides within each of the classes.

Because methylation detection interrogates genomic DNA, but not RNA or protein, it offers several technological advantages in a clinical diagnostic setting: **(1)** readily available source materials. This is particularly important for prognostic research, when only DNA can be reliably extracted from archived paraffin-embedded samples for study; (2) capability for multiplexing, allowing simultaneous measurement of multiple targets to improve assay specificity; (3) easy amplification

of assay products to achieve high sensitivity; (4) robust measurement in tumors that arise from methylation inactivation of one allele of tumor suppressor genes - a process called "functional haploinsufficiency" (Balmain, et al, Nat Genet. 33 Suppl: 238-44 (2003)). It is much easier to detect a methylation change (from negative to positive) than to detect a two-fold gene expression change in these tumors. As such, combining RNA-based gene expression profiling and/or protein-based immunoassays with DNA methylation profiling is contemplated to provide a multi-pronged combination tool that is a sensitive, accurate and robust tool for cancer diagnosis and prognosis (Wong, et al, Curr Oncol Rep. 4(6): 471-7 (2002)).

The present disclosure is directed to a biomarkers and methods using the biomarkers for the identification of differentially methylated CpG dinucleotides within genomic DNA that are particularly informative with respect to the determination, diagnosis or prognosis of ovarian cancer. The biomarkers disclosed herein can be used alone, in combinations of two or more, three or more, four or more, five or more, ten or more, 20 or more, 50 or more, 100 or more or 200 or more, such as components of a gene panel, further in combination with one or more additional diagnostic assays, for diagnostic and/or prognostic assays of ovarian cancer.

In some embodiments, the biomarkers useful in determining, diagnosing or prognosing ovarian cancer in a subject comprise one or more of SEQ ID NO: 1-300. In some embodiments, at least one or more of SEQ ID NO: 1-10 can be included in methods for determining, diagnosing or prognosing ovarian cancer. In one embodiment, biomarkers useful in methods to determine, diagnose or prognose ovarian cancer in a subject comprise one or more of SEQ ID NO: 1-5. In some embodiments, one or more of SEQ ID NO: 6-10 can be further included in ovarian cancer diagnostic or prognostic methods. In further embodiments, one or more of SEQ ID NO: 11- 300 are included in methods in conjunction with one or more of SEQ ID NO: 1-10. In some embodiments, a plurality of any number of biomarkers of SEQ ID NO: 1-300 can be used in methods and assays for ovarian cancer diagnostics and prognostics. A plurality can be two or more, three or more, four or more, five or more, six or more, seven or more, eight or more, nine or more, ten or more, 15 or

more, 20 or more, 30 or more, 50 or more, 75 or more, 85 or more, 100 or more 200 or more or all 300 biomarkers as disclosed herein, or any subset thereof.

In some embodiments, methods for determining the presence of ovarian cancer comprise comparison of genomic DNA from a test sample, for example a sample suspected of having ovarian cancer such as a tissue or cell sample with genomic DNA from a normal tissue, or reference, sample, for example tissue that does not have ovarian cancer. In such determinations, a genomic DNA sample from the test, or suspect tissue, is assayed for methylation status at one or more CpG dinucleotides by any of a number of methods as described herein. A genomic DNA reference normal sample is also assayed and methylation status at one or more CpG dinucleotides of the reference sample is obtained. The methylation status (i.e. level) of the genomic DNA sample at one or more CpG dinucleotides can then be compared to that of the reference sample, and the presence of ovarian cancer determined. For example, biomarker methylation can be decreased relative to a reference sample or increased relative to a reference sample, thereby indicating the presence of ovarian cancer in that patient sample. As disclosed herein, those methylation biomarkers that demonstrate a decreased level of methylation at a particular CpG dinucleotide as compared to the level of methylation at the same location in a reference sample include SEQ ID NO: 1-2, 10, 13-14, 17-18, 21, 28, 30-31, 34, 41-42, 46, 51, 54-58, 63, 65-66, 73, 80-81, 83, 88, 91-96, 98-99, 104-105, 110, 115-116, 125-126, 130-131, 133, 136, 138-139, 143-145, 147-150, 153, 157, 159-160, 163, 166, 168-169, 171, 175, 178-180, 183, 185, 187-189, 197, 200, 202, 204, 212-213, 215-216, 223-225, 231-233, 239, 241, 245-247, 250-252, 254, 256-260, 264-266, 268, 271-272, 274, 276-277, 280-287, 289, 290, 292, 297 and 299-300. Those methylation biomarkers that demonstrate an increased level of methylation at a particular CpG dinucleotide relative to the level in a reference sample include SEQ ID NO: 3-9, 11-12, 15-16, 19-20, 22-27, 29, 32-33, 35-40, 43-45, 47-50, 52-53, 59-62, 64, 67-72, 74-79, 82, 84-87, 89-90, 97, 100-103, 106-109, 111-114, 117-124, 127-129, 432, 134-135, 137, 140-142, 146, 151-152, 154-156, 158, 161-162, 164-165, 167, 170, 172-174, 176-177, 181-182, 184,186, 190-196, 198-199, 201, 203, 205-211, 214, 217-222, 226-230, 234-238, 240, 242-244, 148-149, 253, 255, 261-263, 267, 269-270, 273, 275, 278-279, 288, 291, 293-296 and 298.

In one embodiment, a method for determining the presence of ovarian cancer in a sample from an individual comprises evaluating the methylation status of CpG dinucleotide sequences of a gene or gene region. In some embodiments, evaluating CpG dinucleotide methylation status in one or more of ALX3, ALX4, APRT, AQPl 1, BARX1, C10RF96, C1QL4, C1QTNF4, C1QTNF8, C2ORF60, CACNA1A, CACNA1H, CADPS, CBLN1, CBX2, CD8A, CDH4, CKDN2A, CLCC1, CNST, CNTN5, CREB5, CYP26A1, DDX21, DKK3, DMRTA2, ELFNl, EMX20S, FADD, FAM188B, FBX07, FOXD4L1, FOXM1, GPR158, GPR25, GRIK2, GRXCR2, GSTP1, GUCA1A, HELLS, HLX, IMMP2L, IRX1, IRX2, JPH4, KCND3, KCNS1, KCNTl, KLHL8, KNDCl, KPNA5, LlTDl, LOC494141, LPAR5, MCCC2, MDCl, MELK, MGC13005, MGC2752, MMP23, MOSPD3, MY07A, MYT1L, NFKBIL2, NKAΓN3, NKAPL, NKX2, NKX6, NLGN2, NRN1, NRXN2, PARP14, PAX6, one or more PCDH genes or gene clusters comprising PCDHGA2, A4, Bl, PCDHA6, PCDHB10, Bl 1, B18, B19P and PCDHB2, PEX19, PIGB, POLR2D, PRAME, PRKAG2, PRRT1, PTPRN2, PTPRS, RCC2, RELB, RPL30, RYR1, SFT2D2, SLC25A2, SLC25A33, SLC26A5, SLFN12L, SOX1, SRCIN1, STIM2, SULT1A1, TALI, TBPL2, TBX15, TBX5, TCL1B, TUBB, VENTX, VRK1, WISP1, ZC3H4, ZIC1, ZIC4, ZNF154, ZNF274, ZNF321, ZNF517 and ZNF572 is used in methods to determine the presence of ovarian cancer in a sample. In some embodiments, evaluating CpG dinucleotide methylation status in two or more or CACNA1H, CDH4, ELFNl, EMX20S, GSTP1, GUCA1A, IRX2, KCND3, MDCl, MELK, MMP23, MYT1L, NKAPL, NKX2, NKX6, PCDH, PRAME, PTPRN2, SFT2D2, SULT1A1, TBPL2, TUBB, ZIC4, ZNF154 and ZNF274 is used in methods to determine the presence of ovarian cancer in a sample.

In some embodiments, a method for determining the presence of ovarian cancer in a sample from an individual comprises evaluating the methylation status of CpG dinucleotide sequences in one or more regions not associated with a particular gene. For example, evaluating CpG dinucleotide sequences in one or more regions not associated with a gene or gene region such as those identified in Table 2, is used in methods to determine the presence of ovarian cancer in a sample. In preferred embodiments, methods for determining the presence of ovarian cancer in a sample from an individual comprise evaluating the methylation status of one or more CpG dinucleotide sequences of a gene or gene region, and one or more CpG dinucleotide

sequences of non-gene related region(s). The CpG dinucleotide sequences useful in methods described herein are identified in Table 2.

EXAMPLES

The following examples are provided in order to demonstrate and further illustrate certain preferred embodiments and aspects of the present disclosure and are not to be construed as limiting the scope thereof.

EXAMPLE 1-Study population, sample collection and processing

Samples used for the DNA methylation study included 18 normal ovarian tissue samples (four of them were pooled samples), 21 Fresh-frozen ovarian tumor samples (FF), 67 Formalin-Fixed, Paraffin-Embedded (FFPE) ovarian tumor samples and 37 normal tissue samples from various sources (e.g., adipose, bladder, blood, brain, breast, diaphragm, duodenum, heart, kidney, liver, lung, lymph node, pancreas, muscle, skin, spleen, stomach, testis and ureter). Assay reproducibility was analyzed and verified with more than 20 samples. Ovarian tumor and normal samples are listed in Table 1. The DNA methylation patterns described herein were identified from a group of 21 ovarian cancers compared to normal reference controls (i.e., no diagnosis of ovarian cancer). The cancer tissue samples were categorized, with ovarian cancer subtypes represented for both FF and FFPE tissue samples including those classified as endometroid, serous, mucinous, and clear cell type cancers. The fresh frozen ovarian cancer tissue samples were from a patient cohort previously diagnosed with ovarian cancer under approved protocol from the Internal Review Board at the Mayo Foundation for Medical Education and Research, Rochester, MN.

In preparing the isolated genomic DNA samples for methylation analysis (ILLUMINA Infinium HumanMethylation450 BeadChip microarray assay), isolated genomic DNA was bisulfite treated to convert unmethylated cytosines to uracils while maintaining methylated cytosines using the EZ DNA Methylation Kit (Cat. #D5001, Zymo Research) following manufacturer's recommendations. One of two Infinium assays were performed for each sample, either the ILLUMINA Infinium assay I or II.

For the Infinium I design, two probe/primers per site are used to interrogate CpG loci (the 3' end of the probes are positioned directly across from the CpG site) whereas for Infinium II only one probe/primer is used for each CpG locus (the 3' end of the probe is positioned immediately adjacent the CpG site). Briefly, 500ng of genomic DNA was denatured by addition of Zymo M-Dilution buffer (comprising NaOH) and incubated for 15 minutes at 37°C. Bisulphate containing CT-Conversion reagent was added to the denatured DNA samples and the sample were incubated for 16 hours at 50°C in a thermocycler. During the incubation time the samples were denatured every 60 minutes by heating to 95°C for 30 seconds. Following incubation, the methylated DNA was prepared for microarray analysis following manufacturer's protocol (Infinium HD Assay for Methylation, ILLUMINA, Inc.).

After bisulfite conversion, the FFPE samples were processed using Infinium HD DNA restoration protocol (Part # 15014614 Rev C, ILLUMINA, Inc.). The Infinium HD DNA Restoration protocol restores degraded FFPE DNA to a state that is amplifiable by the Infinium HD FFPE methylation whole genome amplification protocol. Bisulfite converted FF genomic DNA samples or bisulfite converted and restored FFPE genomic DNA samples were processed using Infinium methylation assay. The assay was carried out as described in the Infinium HD Methylation Assay Guide (Part #15019519 Rev B, ILLUMINA, Inc.). Bisulfite-converted DNA (+/- restoration) was used in the whole-genome amplification (WGA) reaction. After amplification, the DNA was enzymatically fragmented, precipitated and re-suspended in a hybridization buffer. All subsequent steps were performed following the standard Infinium protocol (User Guide part #15019519 B). Fragmented DNA was dispensed onto the HumanMethylation450 BeadChips, and hybridization performed in hybridization oven. After hybridization, the array was processed through a primer extension and an immunohistochemistry staining protocol to allow detection of a single-base extension reaction. Finally, BeadChips were coated and imaged on an Illumina iScan.

Briefly, the bisulfite converted DNA was denatured and neutralized in preparation of amplification. Multi-sample amplification mix (MAI) was mixed with DNA in wells of a 96 well plate, denatured with 0.1 N NaOH and neutralized with the addition of random primer mix (RPM) and multi-sample amplification master mix (MSM) to create single stranded DNA for amplification. The denatured and degraded

DNA was isothermally amplified by incubating the samples between 20-24 hrs at 37°C in a hybridization oven. Following incubation, the bisulfite converted and amplified DNA was fragmented. Fragmentation solution (FMS) was added to each sample and the samples were incubated for approximately 1 hr at 37°C. Following sample fragmentation, the DNA was precipitated by the addition of precipitation solution (PM1) and 100% 2-propanol, incubated at 4°C for approximately 30 minutes and centrifuged to pellet the precipitated DNA. The DNA pellets were dried at room temperature.

The precipitated, dried sample DNA was resuspended in RAl (resuspension, hybridization and wash solution), the 96 well plate tightly sealed and the samples incubated in a hybridization oven for 1 hr at 48°C in preparation for hybridizing the fragmented DNA to the HumanMethylation450 BeadChip. Humidifying buffer (PB2) was added to the buffer reservoirs in the assembled hybridization chambers and the BeadChips were correctly oriented into the chamber for barcode reading. The 96 well plate containing the fragmented sample DNA was heated to 95°C for 20 min to denature the sample DNA fragments. Following denaturation, samples were loaded onto the appropriate BeadChip section and visualization confirmed sample dispersion over the entire associated BeadChip section. The hybridization chamber was closed and incubated in a hybridization oven at 48°C for 20 hours. The BeadChips were washed by repeated submersion in the wash buffer PB 1 in preparation for processing the BeadChip for subsequent imaging.

The washed BeadChips with the hybridized fragmented sample DNA were placed onto the Multi-sample BeadChip Alignment Fixture (prefilled with PB1), submersed in PBlsuch that the BeadChip barcodes were correctly oriented and a spacer placed on top of each BeadChip. Following alignment, a glass back plate was placed on the spacer of each BeadChip and the BeadChip/back plate was clamped together with metal clamps to create a flow-through BeadChip chambers for subsequent DNA extension and labeling.

A Chamber Rack which can hold multiple flow-through BeadChip chambers was used for extension and labeling of the DNA immobilized on the BeadChip. For single-base extension of the immobilized fragmented DNA on the BeadChip, the BeadChip chambers were placed into a preheated 44°C heated Chamber Rack and

RAl solution was added into the reservoir for each BeadChip chamber, followed by the stepwise addition of XStain BeadChip solution 1 (XC1), 10 min incubation, addition of XStain BeadChip solution 2 (XC2), 10 min incubation, addition of Two-color extension master mix (TEM), 15 min incubation, addition of 95% formamide/lmM EDTA solution (2X) and addition of XStain BeadChip solution 3 (XC3). Following single-base extension, labeling of the samples was carried out. Labeling was performed by the stepwise addition and incubation of the BeadChip with Superior Two Color Master Mix (STM)-XStain BeadChip solution 3 (XC3)-Anti-Stain Two-Color Master Mix (ATM)-XC3 for a total of two and one-half times. The BeadChip chambers were dismantled and the BeadChips washed sequentially with PB1 and XStain BeadChip solution 4 (XC4), vacuum dried and placed in a desiccator until use.

EXAMPLE 2-DNA methylation marker selection

DNA methylation of CpG dinucleotide sequences were identified using the DNA from ovarian cancer patient samples, known healthy ovarian tissue samples and DNA from non-ovarian, non-cancerous tissue samples as described in Example 1. The DNA methylation status at approximately 500,000 loci was determined. The BeadChips were imaged using the iScan Reader (ILLUMINA, Inc.) and data analyzed using the GENOMESTUDIO Methylation Module (ILLUMINA, Inc.) software following manufacturer's protocol (GENOMESTUDIO Methylation Module vl.8).

For each locus, the median beta value was calculated for each of the three tissue types. The beta value reflects the methylation status of each CpG locus, ranging from 0 in the cases of completely unmethylated sites to 1 is completely methylated sites. The absolute difference in median beta was computed between the cancerous and normal ovarian samples. The absolute difference in median beta was also computed between the cancerous and non-ovarian tissue. The minimum of the two values represents the difference metric. All loci with a difference metric below 0.2 were excluded from further analysis. Following exclusion, 12,929 loci had a difference metric above 0.2. For subsequent analysis, the healthy ovarian and non-ovarian tissues were grouped together and labeled "non-cancerous." At each remaining locus a beta value was determined, that when used as a beta value

threshold, minimized misclassification of cancerous and non-cancerous samples. The 12,929 loci were ranked with respect to their misclassification rate. If there were loci of equal rank, those loci were ranked using the reciprocal of the previously computed difference metric of the loci.

Using the ranked list, the top *n* loci (for example, 300 markers) were used to classify the loci. The beta value calculated for each of the *n* markers was used to classify a new sample, using the optimum beta-value threshold for each marker thereby resulting in *n* classifications. A summary metric was computed as the percentage of markers that classify the new sample as "cancerous" as opposed to "non-cancerous". For example, if 30 out of 300 markers classified the sample as "cancerous", the Methylation sample score would be 0.1 and the sample would be classified as "non-cancerous", using 0.2 as the cutoff score. Conversely, if 150 markers classified the sample as "cancerous", the Methylation sample score would be 0.5 and the sample would be classified as "cancerous". It was determined that a threshold of 0.2 would accurately distinguish the sample classes.

The *n*-marker set of loci was evaluated on an independent set of seventy-six FFPE ovarian cancer samples, 9 healthy ovarian samples, and 14 non-ovarian samples. To compare different values of *n*, receiver-operator-characteristic (ROC) curves were computed and compared. As the ROC curve reports the sensitivity (true positive rate) versus the false positive rate (1-specificity) for a binary classifier system (Metz, 1978, Sem Nuc Med 8:283-298). Figure 1 demonstrates an example when setting *n* equal to one hundred. The accuracy of an assay depends on how well the test samples (i.e., ovarian cancer samples) separate from the control samples (i.e., normal and non-cancerous tissues). As seen in Figure 1, the area is for all intents and purposes 1, thereby representing almost perfect sensitivity and specificity in the classification system utilized herein for discriminating and correctly classifying ovarian cancer from non-ovarian cancer in the sample tissues based on methylation status.

An additional method to further summarize the ROC curve in Figure 1 is by calculating the area under the curve (AUC). This was done for n = 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000 markers and summarized in Figure 2. The AUC was maximized at 300 markers (circle) for

determining ovarian cancer methylation markers as disclosed herein as it is evident from Figure 2 that the area of 300 markers demonstrates excellent sensitivity (AUC~1) for discrimination between ovarian cancer and non-ovarian cancer. However, Figure 2 also demonstrates that as little as 10 of the 300 biomarkers has an AUC of >0.900, as such still an excellent test for diagnostic purposes. The box plots of Figure 3 constructed from summary of the 300 marker data further illustrates the fact that the set and subsets of the 300 markers significantly separate cancerous (tumor) and non-cancerous (normal) tissues.

Of the 300 CpG loci identified as being associated with ovarian cancer 207 methylated CpG dinucleotide islands were associated with 115 genes or gene families and 93 CpG loci were not associated with any particular gene region. The 93 non-gene associated CpG loci were determined to be associated with a number of different genetic features, for example empirically determined differentially methylated regions (10), enhancer regions (16), regulatory regions (13) or DNase I hypersensitive regions (11). Over half of the loci located outside gene regions are associated with CpG islands (42 annotated and 78 hidden Markov Model islands). Table 3 lists the 300 CpG dinucleotide markers (SEQ ID NO: 1-300), the relative position (MAPINFO) on the chromosome (CHR) of the C in the CG dinucleotide based on the Genome build 37 and from which Infinium design (INF, I or II) they were identified.

EXAMPLE 3-Protocadherin gamma gene cluster differentially methylated loci

The following example demonstrations the application of the methods as found in Examples 1 and 2 in determining methylation status in a sample. The DNA samples were prepped as described in Example 1 and microarray analysis was carried out as described in Example 2.

Protocadherin gamma gene cluster is one of three related clusters tandemly linked on chromosome five. The gene cluster has an immunoglobulin-like organization, suggesting that a novel mechanism may be involved in their regulation and expression. The gamma gene cluster includes 22 genes divided into 3 subfamilies. Subfamily A contains 12 genes, subfamily **B** contains 7 genes and 2 pseudogenes, and the more distantly related subfamily C contains 3 genes. The tandem array of 22 large, variable region exons is followed by a constant region, containing 3 exons

shared by all genes in the cluster. Each variable region exon encodes the extracellular region, which includes 6 cadherin ectodomains and a transmembrane region. The constant region exons encode the common cytoplasmic region. These neural cadherin-like cell adhesion proteins are contemplated to play a critical role in the establishment and function of specific cell-cell connections in the brain. Alternative splicing has been described for the gamma cluster genes.

The methylation profile for the protocadherin gamma gene cluster from an ovarian cancer sample and a normal ovarian sample identified 30 CpG loci as differentially methylated between the ovarian tumor and normal ovarian samples as reported in Table 2 (PCDHGnn).

All publications and patents mentioned in the present application are herein incorporated by reference. Various modification and variation of the described methods and compositions of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention that are obvious to those skilled in the relevant fields are intended to be within the scope of the following claims.

CLAIMS

1. A method for identifying the presence of ovarian cancer in an individual comprising:

a) measuring the level of methylation in a nucleic acid test sample from an individual suspected of having ovarian cancer at a CpG dinucleotide sequence in a plurality of genomic targets selected from SEQ ID NOs: 1-300,

b) determining at least a p-value or β-AVE for differential level of methylation at said CpG dinucleotide sequences in the plurality of genomic targets in the nucleic acid test sample relative to a reference level of methylation of said genomic CpG dinucleotide sequences from a normal nucleic acid sample,

c) identifying the presence of ovarian cancer based on said determination.

2. The method of claim 1, wherein said determination results in a combination of genomic targets that identifies ovarian cancer with an AUC of >0.992.2.

3. The method of claim 2, wherein the differential level of methylation identifies those differentially methylated genomic CpG dinucleotide sequences useful for combining as a plurality of genomic targets to diagnose ovarian cancer in an individual.

4. The method of claim 1, wherein said level of methylation in the nucleic acid test sample at one or more of the plurality of CpG dinucleotide sequences is decreased in comparison to the reference level in a normal nucleic acid sample and wherein the level of methylation at one or more of the plurality of CpG dinucleotide sequences in the nucleic acid sample is decreased in SEQ ID NOs: 1-2, 10, 13-14, 17-18, 21, 28, 30-31, 34, 41-42, 46, 51, 54-58, 63, 65-66, 73, 80-81, 83, 88, 91-96, 98-99, 104-105, 110, 115-116, 125-126, 130-131, 133, 136, 138-139, 143-145, 147-150, 153, 157, 159-160, 163, 166, 168-169, 171, 175, 178-180, 183, 185, 187-189, 197, 200, 202, 204, 212-213, 215-216, 223-225, 231-233, 239, 241, 245-247, 250-252, 254, 256-260, 264-266, 268, 271-272, 274, 276-277, 280-287, 289, 290, 292, 297 and 299-300 in comparison to the reference level.

5. The method of claim 1, wherein said level of methylation in the nucleic acid sample is increased at one or more of the plurality of CpG dinucleotide sequences in comparison to the reference level in a normal nucleic acid sample and wherein said level of methylation in the nucleic acid sample is increased in SEQ ID NOs: 3-9, 11-12, 15-16, 19-20, 22-27, 29, 32-33, 35-40, 43-45, 47-50, 52-53, 59-62, 64, 67-72, 74-79, 82, 84-87, 89-90, 97, 100-103, 106-109, 111-114, 117-124, 127-129, 432, 134-135, 137, 140-142, 146, 151-152, 154-156, 158, 161-162, 164-165, 167, 170, 172-174, 176-177, 181-182, 184,186, 190-196, 198-199, 201, 203, 205-211, 214, 217-222, 226-230, 234-238, 240, 242-244, 148-149, 253, 255, 261-263, 267, 269-270, 273, 275, 278-279, 288, 291, 293-296 and 298 in comparison to the reference level.

6. The method of claim 1, wherein differentially methylated genomic CpG dinucleotide sequences are observed in a subset of said genomic targets and wherein said subset comprises two or more of the genomic targets set forth as SEQ ID NOS: 3, 4, 5, 6, 7, 9, 11, 12, 15, 18, 20, 22, 26, 27, 33, 34, 36, 38, 41, 42, 43, 45, 49, 57, 58, 60, 61, 62, 63, 66, 69, 70, 71, 72, 74, 77, 79, 82, 84, 85, 86, 87, 89, 93, 100, 102, 103, 104, 107, 110, 111, 112, 113, 114, 117, 121, 122, 123, 125, 127, 128, 132, 133, 134, 144, 146, 151, 152, 153, 154, 156, 163, 164, 165, 166, 167, 168, 172, 174, 179, 186, 190, 197, 200, 204, 205, 206, 209, 211, 212, 222, 223, 226, 228, 230, 234, 235, 238, 243, 244, 251, 253, 255, 260, 263, 265, 266, 269, 270, 274, 275, 277, 285, 289 and 295.

7. A population of genomic targets selected from the group consisting of SEQ ID NO: 1-300 wherein said population is useful in diagnosing ovarian cancer by demonstrating in their combination an AUC of >0.992 useful in practicing the method of claim 1.

8. The population of genomic targets of claim 7, wherein said targets exhibit differential methylation of genomic CpG dinucleotide sequences in ovarian cancer and wherein said differential methylation is diagnostic for the presence of ovarian cancer.

9. The population of genomic targets of claim 7, further comprising a subset of genomic targets selected from the group consisting of SEQ ID NOs: 3, 4, 5,

6, 7, 9, 11, 12, 15, 18, 20, 22, 26, 27, 33, 34, 36, 38, 41, 42, 43, 45, 49, 57, 58, 60, 61, 62, 63, 66, 69, 70, 71, 72, 74, 77, 79, 82, 84, 85, 86, 87, 89, 93, 100, 102, 103, 104, 107, 110, 111, 112, 113, 114, 117, 121, 122, 123, 125, 127, 128, 132, 133, 134, 144, 146, 151, 152, 153, 154, 156, 163, 164, 165, 166, 167, 168, 172, 174, 179, 186, 190, 197, 200, 204, 205, 206, 209, 211, 212, 222, 223, 226, 228, 230, 234, 235, 238, 243, 244, 251, 253, 255, 260, 263, 265, 266, 269, 270, 274, 275, 277, 285, 289 and 295.

10. The population of genomic targets of claim 8, wherein the differential methylation is decreased methylation of CpG dinucleotide sequences in a test nucleic acid sample from an individual in comparison to a reference level from a normal nucleic acid sample.

11. The population of genomic targets of claim 10, wherein the level of methylation in the sample from an individual is decreased in SEQ ID NOs: 1-2, 10, 13-14, 17-18, 21, 28, 30-31, 34, 41-42, 46, 51, 54-58, 63, 65-66, 73, 80-81, 83, 88, 91-96, 98-99, 104-105, 110, 115-116, 125-126, 130-131, 133, 136, 138-139, 143-145, 147-150, 153, 157, 159-160, 163, 166, 168-169, 171, 175, 178-180, 183, 185, 187-189, 197, 200, 202, 204, 212-213, 215-216, 223-225, 231-233, 239, 241, 245-247, 250-252, 254, 256-260, 264-266, 268, 271-272, 274, 276-277, 280-287, 289, 290, 292, 297 and 299-300 in comparison to a reference level.

12. The population of genomic targets of claim 8, wherein the differential methylation is increased methylation level of said CpG dinucleotide sequences in a test nucleic acid sample from an individual in comparison to a reference level from a normal nucleic acid sample.

13. The population of genomic targets of claim 12, wherein the level of methylation in the nucleic acid sample is increased in SEQ ID NOs: 3-9, 11-12, 15-16, 19-20, 22-27, 29, 32-33, 35-40, 43-45, 47-50, 52-53, 59-62, 64, 67-72, 74-79, 82, 84-87, 89-90, 97, 100-103, 106-109, 111-114, 117-124, 127-129, 432, 134-135, 137, 140-142, 146, 151-152, 154-156, 158, 161-162, 164-165, 167, 170, 172-174, 176-177, 181-182, 184,186, 190-196, 198-199, 201, 203, 205-211, 214, 217-222, 226-230, 234-238, 240, 242-244, 148-149, 253, 255, 261-263, 267, 269-270, 273, 275, 278-279, 288, 291, 293-296 and 298 in comparison to a reference level.

14. The method of claim 1, wherein said plurality of genomic targets is at least 10 genomic targets.

15. The population of genomic targets of claim 7, wherein said population of genomic targets is at least 10 genomic targets.

FIGURE 1

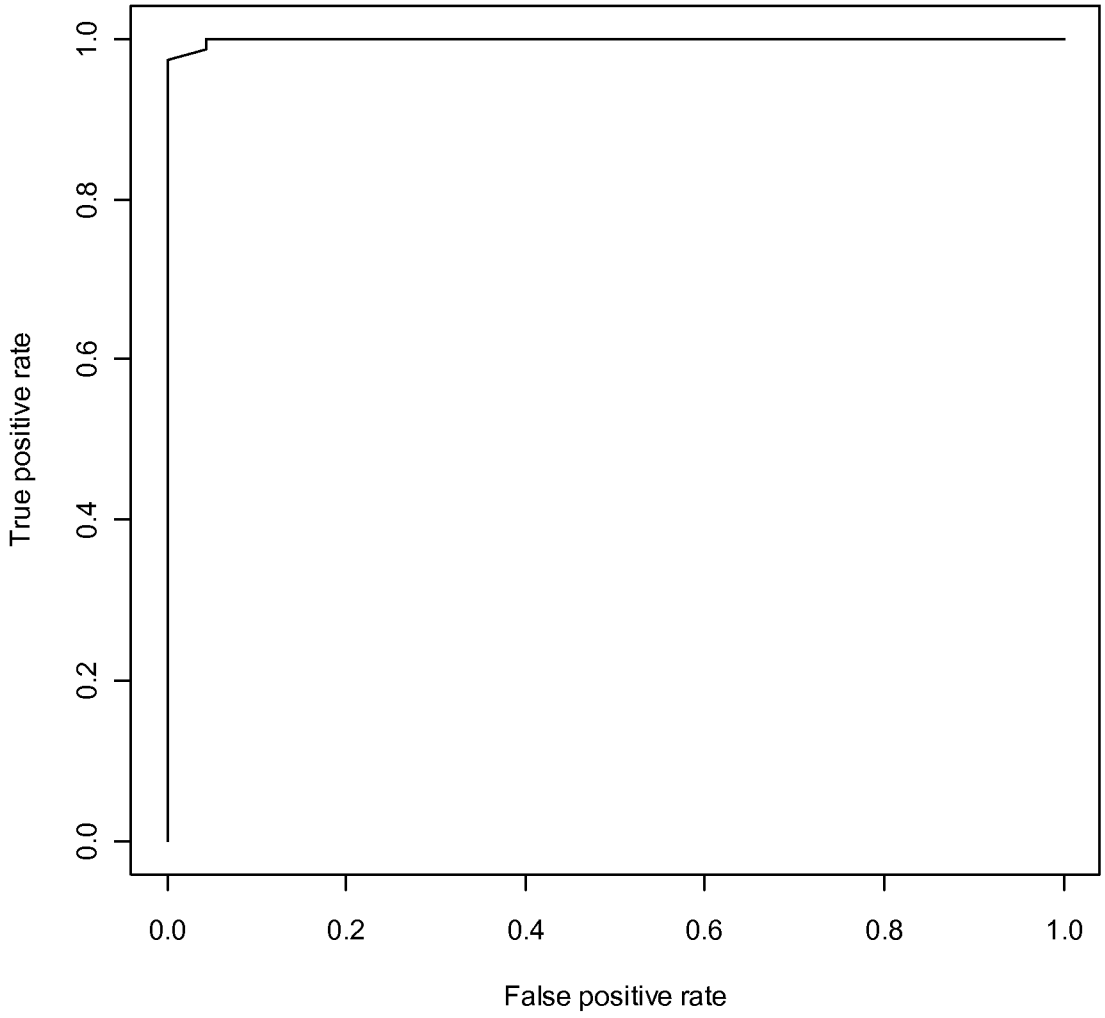

True positive rate
0.0
0.2
0.4
0.6
0.8
1.0
False positive rate
0.0
0.2
0.4
0.6
0.8
1.0

FIGURE 2

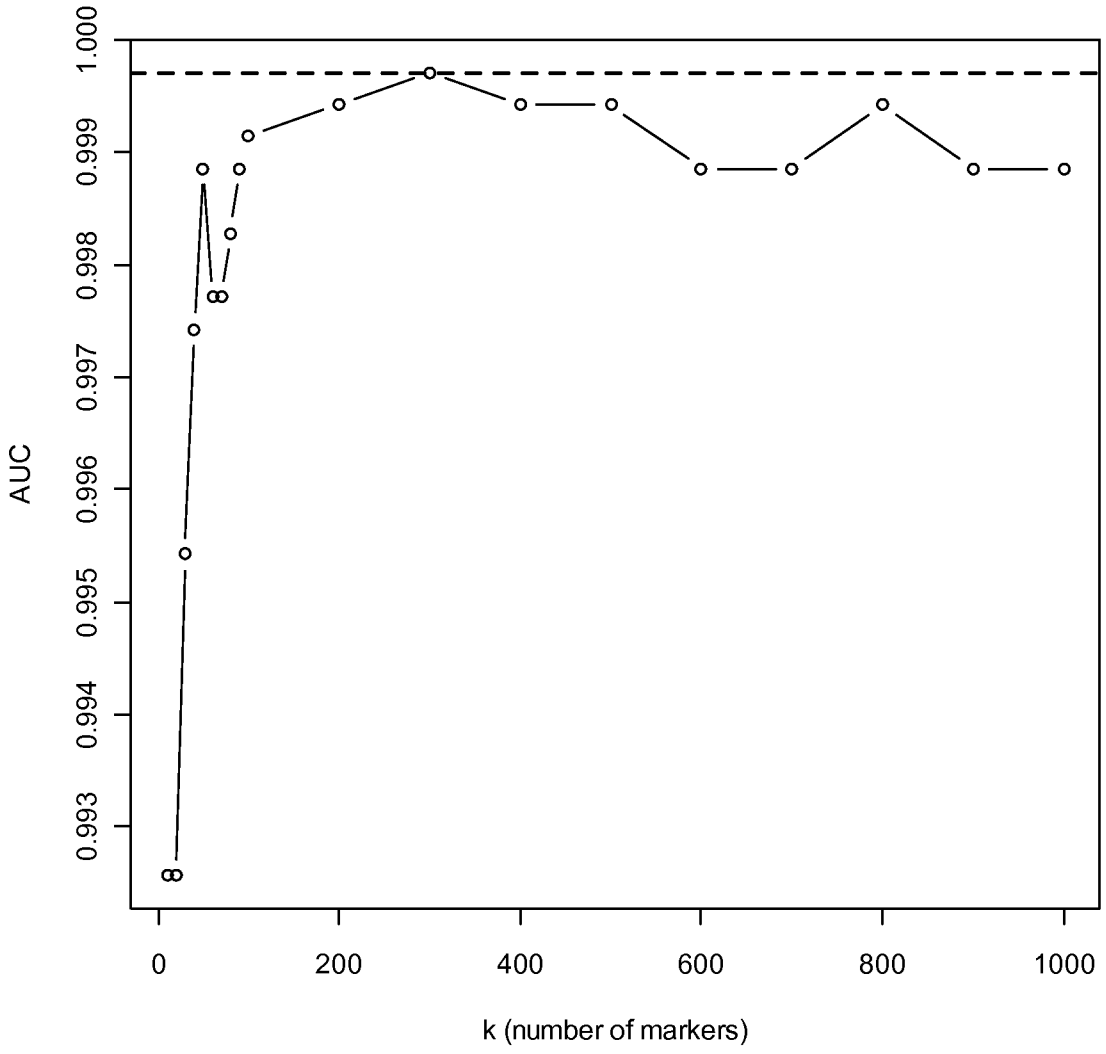
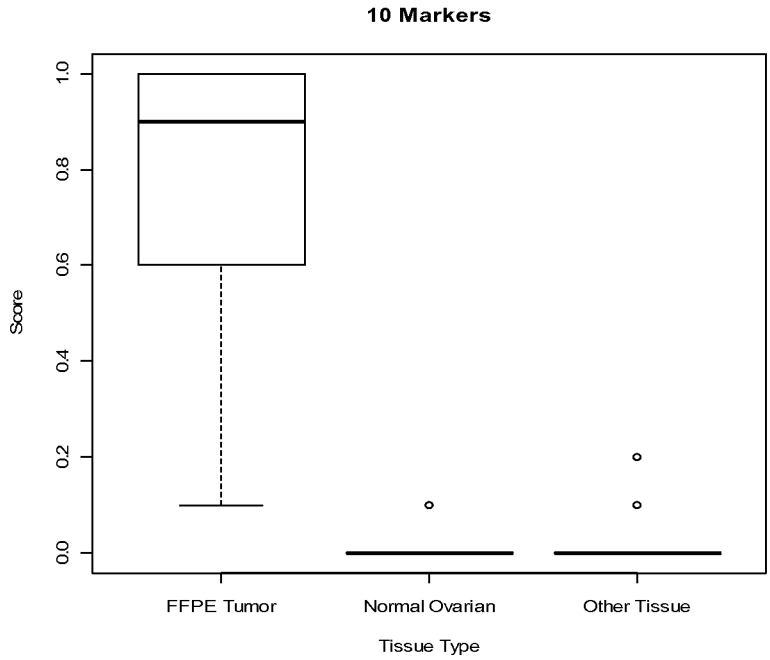

AUC
1.000
0.999
0.998
0.997
0.996
0.995
0.994
0.993
0
200
400
600
800
1000
k (number of markers)

FIGURE 3



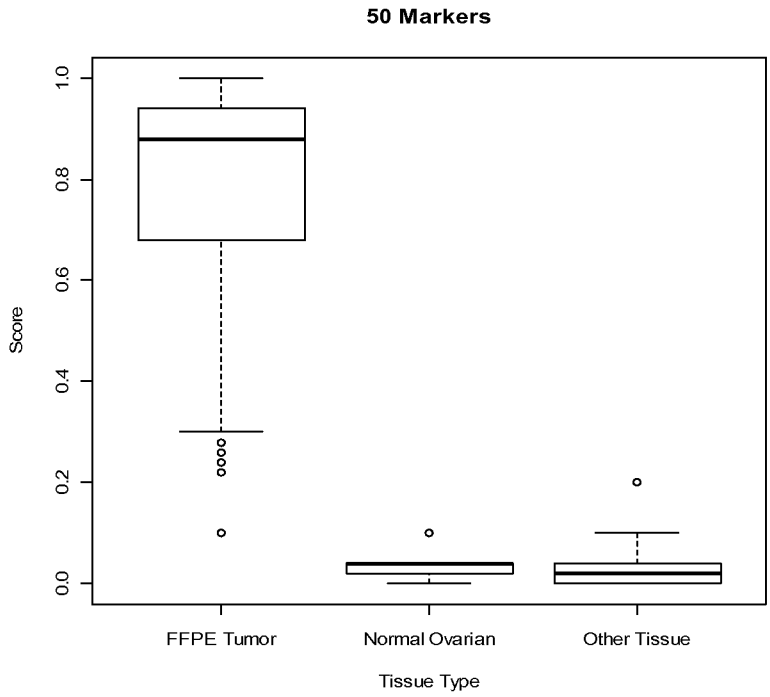
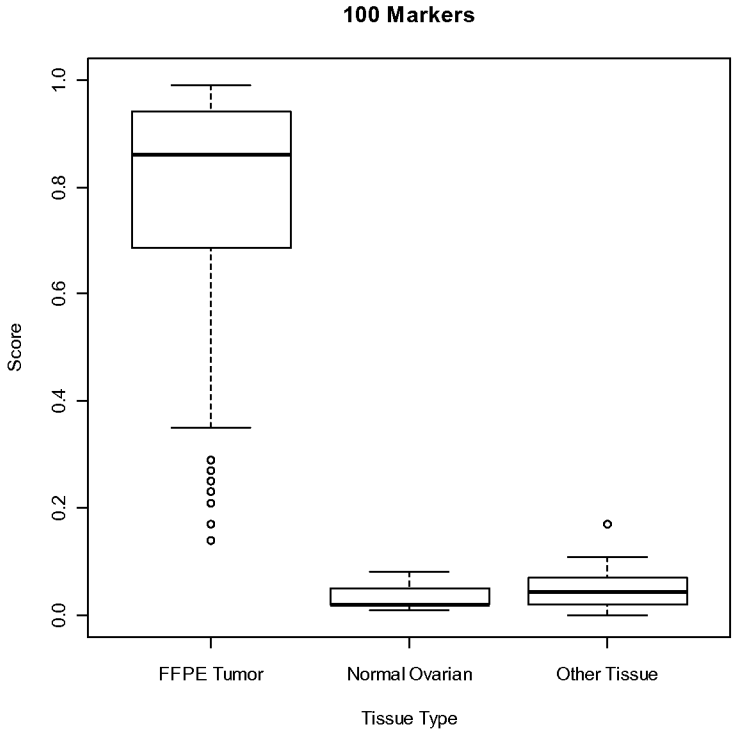


FIGURE 3 (cont.)



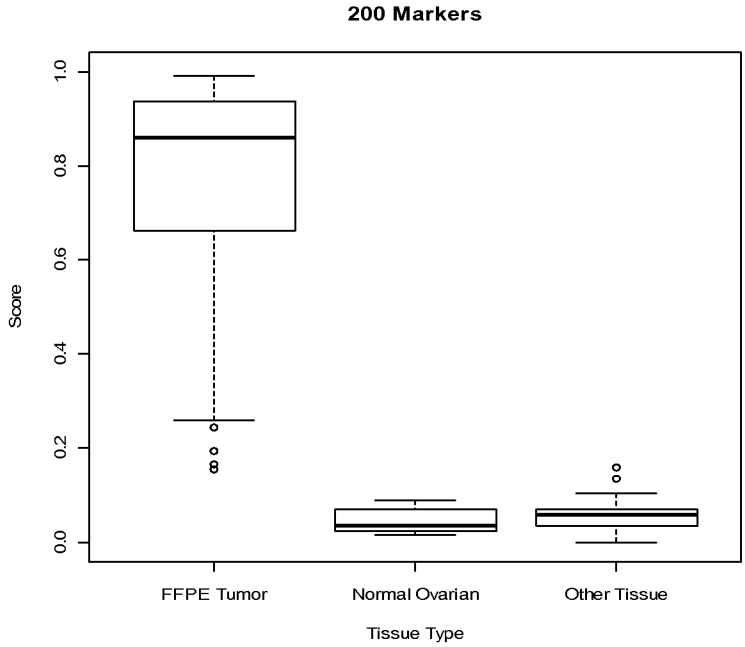
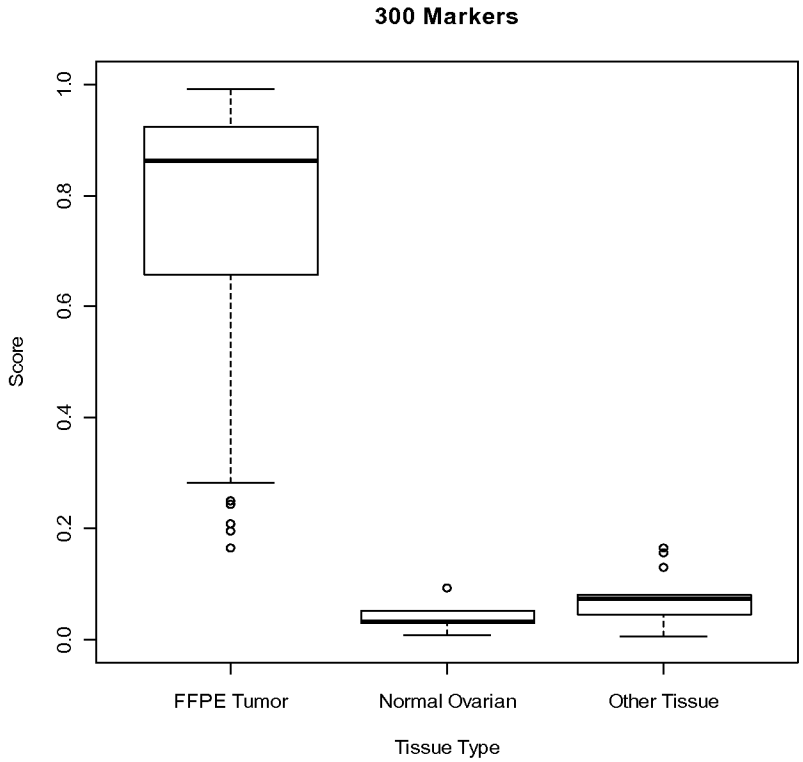


FIGURE 3 (cont.)


300 Markers
Score
0.0
0.2
0.4
0.6
0.8
1.0
FFPE Tumor
Normal Ovarian
Other Tissue
Tissue Type

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/071031

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/68
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal , WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
25 April 2013	07/05/2013

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bruma, Anja

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y	WO 2006/031831 A2 (FOX CHASE CANCER CT [US] ; CAIRNS PAUL G [US]) 23 March 2006 (2006-03-23) claims 1-11 page 11, paragraph 2	1-15
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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No
PCT/US2012/071031

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