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(54) Title: CHARACTERIZING METHYLATED DNA, RNA, AND PROTEINS IN THE DETECTION OF LUNG NEOPLASIA

FIG. 1

AGRN Target DNA (SEQ ID NO:1)

5′GTTCCCGGAACGGCCTCTTGGGGGCGTTCCAGCCCCACGGACCCGCAGGGAGTCCCCGCCGCAATTTGCATGGGG
CTCATTTGCATGACCCCGCCCCGCGCGGGAGTCGGGGGCGC3′

Bisulfite-converted Target DNA: (SEQ ID NO:2)

5′GTTTTCGGAACGGTTTTTTGGGGGCGTTTTAGTTTTACGGATTCGTAGGGAGTTTTCGTCGTAATTTGTATGGGG
TTTATTTGTATGATTTCGTTTCGCGCGGGAGTCGGGGGCGT3′

PCR and Flap Assay Oligonucleotides:

AGRN Forward Primer: 5′ GGCGTTTTAGTTTTACGGATTCG3′ (SEQ ID NO:3)
AGRN Reverse Primer: 5′ ACAAATAAACCCCATACAAATTACGAC3′ (SEQ ID NO:4)
AGRN Flap oligo.: 5′ CGCCGAGGCGAAAACTCCCT/3C6/ (SEQ ID NO:5)

(57) Abstract: Provided herein is technology relating to detecting neoplasia and particularly, but not exclusively, to methods, compositions, and related uses for detecting neoplasms such as lung cancer.

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CHARACTERIZING METHYLATED DNA, RNA, AND PROTEINS IN THE DETECTION OF LUNG NEOPLASIA

The present application claims priority to U.S. Provisional Application Serial No. 62/771,965, filed November 27, 2018, which is incorporated herein by reference.

FIELD OF THE INVENTION

Provided herein is technology relating to detecting neoplasia and particularly, but not exclusively, to methods, compositions, and related uses for detecting neoplasms such as lung cancer.

BACKGROUND OF THE INVENTION

Lung cancer remains the number one cancer killer in the US, and effective screening approaches are desperately needed. Lung cancer alone accounts for 221,000 deaths annually. DNA methylation profiling has shown unique patterns in DNA promoter regions with cancer and has potential application for detection of lung malignancies. However, optimally discriminant markers and marker panels are needed.

SUMMARY OF THE INVENTION

Provided herein is a collection of methylation markers assayed on tissue or plasma that achieves extremely high discrimination for all types of lung cancer while remaining negative in normal lung tissue and benign nodules. Markers selected from the collection can be used alone or in a panel, for example, to characterize blood or bodily fluid, with applications in lung cancer screening and discrimination of malignant from benign nodules. In some embodiments, markers from the panel are used to distinguish one form of lung cancer from another, *e.g.*, for distinguishing the presence of a lung adenocarcinoma or large cell carcinoma from the presence of a lung small cell carcinoma, or for detecting mixed pathology carcinomas. Provided herein is technology for screening markers that provide a high signal-to-noise ratio and a low background level when detected from samples taken from a subject.

Methylation markers and/or panels of markers (*e.g.*, chromosomal region(s)) having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX*

chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1, and *HOPX* were identified in studies by comparing the methylation state of methylation markers from lung cancer samples to the corresponding markers in normal (non-cancerous) samples.

As described herein, the technology provides a number of methylation markers and subsets thereof (*e.g.*, sets of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more markers) with high discrimination for lung cancer and, in some embodiments, with discrimination between lung cancer types. Experiments applied a selection filter to candidate markers to identify markers that provide a high signal to noise ratio and a low background level to provide high specificity and selectivity for purposes of characterizing biological samples, *e.g.*, for cancer screening or diagnosis. For example, as described herein below, analysis of methylation of combination of 8 markers, *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1*, resulted in 98.5% sensitivity (134/136 cancers) for all of the cancer tissues tested, with 100% specificity. In another embodiment, a panel of 6 markers (*SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI*) resulted in a sensitivity of 92.2% at 93% specificity, and a panel of 4 markers (*ZNF781, BARX1, EMX1,* and *HOXA9*) resulted in an overall sensitivity of 96% and specificity of 94%.

Accordingly, provided herein is technology related to a method of processing a sample obtained from a subject, the method comprising assaying a methylation state of one or more marker genes in the sample. In preferred embodiments, the methylation state of the methylation marker is determined by measuring the amounts of a methylation marker and of a reference marker in the sample, and comparing the amount of the methylation marker to the amount of reference marker in the sample to determine a methylation state for the methylation marker in the sample. While not limiting the invention to any particular application or applications, the method finds use, *e.g.*, in characterizing samples from a subject having or suspected of having lung cancer, when the methylation state of the methylation marker is different than a methylation state of that marker assayed in a subject that does not have a neoplasm. In preferred embodiments, the methylation marker comprises a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B,*

DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1, and *HOPX.* In some embodiments, the reference marker is selected from *B3GALT6* DNA and β-actin DNA.

In some embodiments, the technology comprises assaying a plurality of markers, *e.g.*, comprising assaying the methylation states of 2 to 21 markers, preferably 2 to 8 markers, preferably 4 to 6 markers. For example, in some embodiments, the method comprises analysis of the methylation status of two or more markers selected from *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2, EMX1, CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1,* and *SKI.* In some preferred embodiments, the method comprises analysis of the methylation status of a set of markers comprising *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1.* In some embodiments, the method comprises analysis of the methylation status of a set of markers selected from: the group consisting of *ZNF781, BARX1,* and *EMX1*; the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI;* the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1;* the group consisting of *SHOX2, SOBP, ZNF781, BTACT, CYP26C1,* and *DLX4;* and the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI.* In certain embodiments, the at least one methylation marker comprises the group selected from *ZNF781, BARX1,* and *EMX1,* and further comprises *SOBP* and/or *HOXA9.* In other embodiments, the at least one methylation marker comprises a group selected from *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B.*

In some embodiments, the at least one methylation marker comprises one or both of *IFFO1* and *HOPX*, and optionally further comprises one or more marker genes selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR,*

GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1 and *ZNF32.* In certain embodiments, the at least one methylation marker gene consists of at least one of *IFFO1* and *HOPX,* and further comprises one or more of *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B,* while in certain preferred embodiments, the at least one methylation marker gene consists of at least one of *IFFO1* and *HOPX* , and the group *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B.*

The technology is not limited in the methylation state assessed. In some embodiments assessing the methylation state of the methylation marker in the sample comprises determining the methylation state of one base. In some embodiments, assaying the methylation state of the marker in the sample comprises determining the extent of methylation at a plurality of bases. Moreover, in some embodiments the methylation state of the marker comprises an increased methylation of the marker relative to a normal methylation state of the marker. In some embodiments, the methylation state of the marker comprises a decreased methylation of the marker relative to a normal methylation state of the marker. In some embodiments the methylation state of the marker comprises a different pattern of methylation of the marker relative to a normal methylation state of the marker.

In some embodiments, the technology provides a method of generating a record reporting a lung neoplasm in a subject, the method comprising the steps of:

a) assaying a sample from a subject for an amount of at least one methylated methylation marker gene selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX* in a sample obtained from a subject;

b) assaying said sample for an amount of reference marker in said sample;

c) comparing the amount of said at least one methylated methylation marker to the amount of reference marker in said sample to determine a methylation state for said at least one methylation marker in said sample; and

d) generating a record reporting the methylation state for said at least one marker gene in said sample, wherein the methylation state of said methylation marker is indicative of the presence or absence of a lung neoplasm in said subject.

In some embodiments, the technology provides a method of characterizing a sample, comprising:

a) measuring an amount of at least one methylation marker gene in DNA selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX*;

b) measuring the amount of at least one reference marker in the DNA; and

c) calculating a value for the amount of the at least one methylation marker gene measured in the DNA as a percentage of the amount of the reference marker measured in the DNA, wherein the value indicates the amount of the at least one methylation marker DNA measured in the sample.

In some preferred embodiments, the at least one methylation marker gene consists of one to fifteen methylation marker genes.

In some embodiments, amounts of at least two of the markers are measured, and preferably the at least two methylation marker genes are selected from the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2, EMX1 CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1,* and *SKI.* In other embodiments, the methylation markers comprise a

group selected from *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B.*In certain preferred embodiments, the method comprises analysis of the methylation status of a set of markers selected from: the group consisting of *ZNF781, BARX1,* and *EMX1*; the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI;* the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1;* the group consisting of *SHOX2, SOBP, ZNF781, BTACT, CYP26C1,* and *DLX4;* and the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI.* In certain embodiments, the at least one methylation marker comprises the group selected from *ZNF781, BARX1,* and *EMX1,* and further comprises *SOBP* and/or *HOXA9.* In some embodiments, methylation markers are selected such that the methylation status of said one or more markers is indicative of only one of lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, or small cell carcinoma. In other embodiments, methylation markers are selected such that the methylation status of said one or more markers is indicative of more than one of lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and small cell carcinoma. In yet other embodiments, methylation markers are selected such that the methylation status of said one or more markers is indicative of any one of or combination of lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, small cell carcinoma, generic non-small cell lung cancer, and/or undefined lung carcinoma. In some embodiments assaying or measuring the methylation state of the methylation marker in the sample comprises determining the methylation state of one base, while in other embodiments the assay comprises determining the extent of methylation at a plurality of bases. In some embodiments the methylation state of the marker comprises an increased or decreased methylation of the marker relative to a normal methylation state of the marker, *e.g.,* as the marker would appear in a non-cancerous sample, while in some embodiments the methylation state of the marker comprises a different pattern of methylation of the marker relative to a normal methylation state of the marker. In preferred embodiments the reference marker is a methylated reference marker. In some embodiments, the reference marker comprises a portion of a gene.

The technology is not limited to particular sample types. For example, in some embodiments the sample is a tissue sample, a blood sample, a plasma sample, a serum sample, or a sputum sample. In certain preferred embodiments a tissue sample comprises lung tissue. In certain preferred embodiments, the sample comprises DNA isolated from plasma.

The technology is not limited to any particular method of assaying DNA from samples. For example, in some embodiments the assaying comprises using polymerase chain reaction, nucleic acid sequencing, mass spectrometry, methylation specific nuclease, mass-based separation, and/or target capture. In certain preferred embodiments the assaying comprises using a flap endonuclease assay.

In some embodiments, the DNA is treated with a reagent that selectively modifies DNA in a manner specific to the methylation status of the DNA. For example, in in some embodiments, DNA is treated with a restriction enzyme that a methylation-sensitive restriction enzyme, or a methylation-dependent restriction enzyme.

In particularly preferred embodiments the sample DNA and/or reference marker DNA are bisulfite-converted and the assay for determining the methylation level of the DNA is achieved by a technique comprising the use of methylation-specific PCR, quantitative methylation-specific PCR, methylation-specific DNA restriction enzyme analysis, quantitative bisulfite pyrosequencing, flap endonuclease assay (*e.g.*, a QUARTS flap endonuclease assay), and/or bisulfite genomic sequencing PCR.

The technology also provides methods of characterizing a sample or combination of samples from a subject comprising analyzing the sample(s) for a plurality of different types of marker molecules. For example, in some embodiments, the technology provides a method comprising measuring an amount of at least one methylation marker gene in DNA from a sample obtained from a subject, and further comprises one or more of measuring an amount of at least one RNA marker in a sample obtained from the subject, and assaying for the presence or absence of at least one protein marker in a sample obtained from the subject. In some embodiments, a single sample from a subject is analyzed for methylation marker DNA(s), marker RNA(s), and marker protein(s).

Analysis of DNA, RNA and protein markers are not limited to use of any particular technologies. Methods for analyzing DNA and RNA are well known, in include but are not limited to nucleic acid detection assays comprising amplification, probe hybridization, for example. Methods for analyzing proteins include but are not limited to enzyme-linked immunosorbent assay (ELISA) detection, protein immunoprecipitation, Western blot, immunostaining, *etc.*

The technology also provides kits. For example, in some embodiments the technology provides a kit, comprising a) at least one oligonucleotide, wherein at least a portion of the

oligonucleotide specifically hybridizes to a marker selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX.* In preferred embodiments, the portion of the oligonucleotide that hybridizes to the marker specifically hybridizes to bisulfite-treated DNA comprising the methylation marker. In some embodiments, the kit comprises at least one additional oligonucleotide, wherein at least a portion of the additional oligonucleotide specifically hybridizes to a reference nucleic acid. In some embodiments the kit comprises at least two additional oligonucleotides and, in some embodiments, the kit further comprises a bisulfite reagent.

In certain embodiments at least a portion of the oligonucleotide specifically hybridizes to a least one the marker selected from the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2, EMX1, CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1,* and *SKI.* In other embodiments, at least a portion of the oligonucleotide specifically hybridizes to a least one the marker selected from the group consisting of *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B.*

In preferred embodiments, the kit comprises a set of oligonucleotides, each of which hybridizes to one marker in a set of markers, the set of markers selected from: the group consisting of *ZNF781, BARX1,* and *EMX1*; the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI;* the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1;* the group consisting of *SHOX2, SOBP, ZNF781, BTACT, CYP26C1,* and *DLX4;* and the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI.* In certain embodiments, the set of methylation markers comprises the group selected from *ZNF781, BARX1,* and *EMX1,* and further comprises *SOBP* and/or *HOXA9.* In some embodiments, the set of markers comprises one or both of *IFFO1* and *HOPX*, and further comprises one or more markers selected from the

group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1* and *ZNF32.* In other embodiments, the set of methylation markers comprises one or both of *IFFO1* and *HOPX*, and further comprises one or more markers selected from *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B.* In certain embodiments, the set of methylation markers consists of one or both of *IFFO1* and *HOPX*, and one or more markers selected from *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B.*

In some embodiments, the at least one oligonucleotide in the kit is selected to hybridize to methylation marker(s) that are indicative of only one of type of lung carcinoma, *e.g.*, lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, or small cell carcinoma. In other embodiments, the at least one oligonucleotide is selected to hybridize to methylation marker(s) that are indicative of more than one of lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and small cell carcinoma. In yet other embodiments, the at least one oligonucleotide is selected to hybridize to methylation marker(s) that are indicative of any one of, or any combination of lung adenocarcinoma, large cell carcinoma, squamous cell carcinoma, small cell carcinoma, and/or undefined lung carcinoma.

In preferred embodiments, oligonucleotide(s) provided in the kit are selected from one or more of a capture oligonucleotide, a pair of nucleic acid primers, a nucleic acid probe, and an invasive oligonucleotide. In preferred embodiments, oligonucleotide(s) specifically hybridize to bisulfite-treated DNA comprising said methylation marker(s).

In some embodiments the kit further comprises a solid support, such a magnetic bead or particle. In preferred embodiments, a solid support comprises one or more capture reagents, *e.g.*, oligonucleotides complementary said one or more markers genes.

The technology also provides compositions. For example, in some embodiments the technology provides a composition comprising a mixture, *e.g.*, a reaction mixture, that comprises a complex of a target nucleic acid selected from the group consisting of *BARX1,*

LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12a, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1, and *HOPX,* and an oligonucleotide that specifically hybridizes to the target nucleic acid. In some embodiments, the target nucleic acid is bisulfite-converted target nucleic acid. In preferred embodiments, the mixture comprises a complex of a target nucleic acid selected from the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2, EMX1, CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1,* and *SKI,* and an oligonucleotide that specifically hybridizes to the target nucleic acid (whether unconverted or bisulfite-converted). In other preferred embodiments, the mixture comprises a complex of a target nucleic acid selected from the group consisting of *BARX1, HOXB2, FLJ45983, IFFO1, HOPX, TRH, HOXA9, SOBP, ZNF781,* and *FAM59B,* and an oligonucleotide that specifically hybridizes to the target nucleic acid (whether unconverted or bisulfite-converted). Oligonucleotides in the mixture include but are not limited to one or more of a capture oligonucleotide, a pair of nucleic acid primers, a hybridization probe, a hydrolysis probe, a flap assay probe, and an invasive oligonucleotide.

In some embodiments, the target nucleic acid in the mixture comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 28, 33, 38, 43, 48, 53, 58, 63, 68, 73, 78, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 214, 219, 224, 229, 234, 239, 247, 252, 257, 262, 267, 272, 277, 282, 287, 292, 298, 303, 308, 313, 319, 327, 336, 341, 346, 351, 356, 361, 366, 371, 384, 403, 412, and 426, and complements thereof.

In some embodiments, the mixture comprises bisulfite-converted target nucleic acid that comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 2, 7, 12, 17, 22, 29, 34, 39, 44, 49, 54, 59, 64, 69, 74, 79, 87, 92, 97, 102, 107, 112, 117, 122, 127, 132, 137, 142, 147, 152, 157, 162, 167, 172, 177, 182, 187, 192, 197, 202, 210, 215, 220, 225, 230, 235, 240, 248, 253, 258, 263, 268, 273, 278, 283, 288, 293, 299, 304, 309,

314, 320, 328, 337, 342, 347, 352, 357, 362, 367, 372, 385, 404, 413, and 427, and complements thereof.

In some embodiments, a kit comprises reagents or materials for at least two assays, wherein the assays are selected from measuring an amount of, or the presence or absence of 1) at least one methylated DNA marker; 2) at least one RNA marker; and 3) at least one protein marker. In preferred embodiments, the at least one methylated DNA marker is selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12a, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX.* In some embodiments, the at least one protein comprises an antigen, *e.g.*, a cancer-associated antigen, while in some embodiments, the at least one protein comprises an antibody, *e.g.*, an autoantibody to a cancer-associated antigen.

In some embodiments, an oligonucleotide in said mixture comprises a reporter molecule, and in preferred embodiments, the reporter molecule comprises a fluorophore. In some embodiments the oligonucleotide comprises a flap sequence. In some embodiments the mixture further comprises one or more of a FRET cassette; a FEN-1 endonuclease and/or a thermostable DNA polymerase, preferably a bacterial DNA polymerase.

DEFINITIONS

To facilitate an understanding of the present technology, a number of terms and phrases are defined below. Additional definitions are set forth throughout the detailed description.

Throughout the specification and claims, the following terms take the meanings explicitly associated herein, unless the context clearly dictates otherwise. The phrase "in one embodiment" as used herein does not necessarily refer to the same embodiment, though it may. Furthermore, the phrase "in another embodiment" as used herein does not necessarily refer to a different embodiment, although it may. Thus, as described below, various

embodiments of the invention may be readily combined, without departing from the scope or spirit of the invention.

In addition, as used herein, the term "or" is an inclusive "or" operator and is equivalent to the term "and/or" unless the context clearly dictates otherwise. The term "based on" is not exclusive and allows for being based on additional factors not described, unless the context clearly dictates otherwise. In addition, throughout the specification, the meaning of "a", "an", and "the" include plural references. The meaning of "in" includes "in" and "on."

The transitional phrase "consisting essentially of" as used in claims in the present application limits the scope of a claim to the specified materials or steps "and those that do not materially affect the basic and novel characteristic(s)" of the claimed invention, as discussed in *In re Herz, 537 F.2d 549, 551-52, 190 USPQ 461, 463 (CCPA 1976)*. For example, a composition "consisting essentially of" recited elements may contain an unrecited contaminant at a level such that, though present, the contaminant does not alter the function of the recited composition as compared to a pure composition, *i.e.*, a composition "consisting of" the recited components.

As used herein, "methylation" refers to cytosine methylation at positions C5 or N4 of cytosine, the N6 position of adenine, or other types of nucleic acid methylation. In vitro amplified DNA is usually unmethylated because typical in vitro DNA amplification methods do not retain the methylation pattern of the amplification template. However, "unmethylated DNA" or "methylated DNA" can also refer to amplified DNA whose original template was unmethylated or methylated, respectively.

Accordingly, as used herein a "methylated nucleotide" or a "methylated nucleotide base" refers to the presence of a methyl moiety on a nucleotide base, where the methyl moiety is not present in a recognized typical nucleotide base. For example, cytosine does not contain a methyl moiety on its pyrimidine ring, but 5-methylcytosine contains a methyl moiety at position 5 of its pyrimidine ring. Therefore, cytosine is not a methylated nucleotide and 5-methylcytosine is a methylated nucleotide. In another example, thymine contains a methyl moiety at position 5 of its pyrimidine ring; however, for purposes herein, thymine is not considered a methylated nucleotide when present in DNA since thymine is a typical nucleotide base of DNA.

As used herein, a "methylated nucleic acid molecule" refers to a nucleic acid molecule that contains one or more methylated nucleotides.

As used herein, a "methylation state", "methylation profile", and "methylation status" of a nucleic acid molecule refers to the presence of absence of one or more methylated nucleotide bases in the nucleic acid molecule. For example, a nucleic acid molecule containing a methylated cytosine is considered methylated (*e.g.*, the methylation state of the nucleic acid molecule is methylated). A nucleic acid molecule that does not contain any methylated nucleotides is considered unmethylated. In some embodiments, a nucleic acid may be characterized as "unmethylated" if it is not methylated at a specific locus (*e.g.*, the locus of a specific single CpG dinucleotide) or specific combination of loci, even if it is methylated at other loci in the same gene or molecule.

The methylation state of a particular nucleic acid sequence (*e.g.*, a gene marker or DNA region as described herein) can indicate the methylation state of every base in the sequence or can indicate the methylation state of a subset of the bases (*e.g.*, of one or more cytosines) within the sequence, or can indicate information regarding regional methylation density within the sequence with or without providing precise information of the locations within the sequence the methylation occurs. As used herein, the terms "marker gene" and "marker" are used interchangeably to refer to DNA (or other sample components) that is associated with a condition, *e.g.*, cancer, regardless of whether the marker region is in a coding region of DNA. Markers may include, *e.g.*, regulatory regions, flanking regions, intergenic regions, *etc.* Similarly, the term "marker" used in reference to any component of a sample, *e.g.*, protein, RNA, carbohydrate, small molecule, *etc.*, refers to a component that can be assayed in a sample (*e.g.*, measured or otherwise characterized) and that is associated with a condition of a subject, or of the sample from a subject. The term "methylation marker" refers to a gene or DNA in which the methylation state of the gene or DNA is associated with a condition, *e.g.*, cancer.

The methylation state of a nucleotide locus in a nucleic acid molecule refers to the presence or absence of a methylated nucleotide at a particular locus in the nucleic acid molecule. For example, the methylation state of a cytosine at the 7th nucleotide in a nucleic acid molecule is methylated when the nucleotide present at the 7th nucleotide in the nucleic acid molecule is 5-methylcytosine. Similarly, the methylation state of a cytosine at the 7th nucleotide in a nucleic acid molecule is unmethylated when the nucleotide present at the 7th nucleotide in the nucleic acid molecule is cytosine (and not 5-methylcytosine).

The methylation status can optionally be represented or indicated by a "methylation value" (*e.g.*, representing a methylation frequency, fraction, ratio, percent, etc.) A

methylation value can be generated, for example, by quantifying the amount of intact nucleic acid present following restriction digestion with a methylation dependent restriction enzyme or by comparing amplification profiles after bisulfite reaction or by comparing sequences of bisulfite-treated and untreated nucleic acids. Accordingly, a value, *e.g.*, a methylation value, represents the methylation status and can thus be used as a quantitative indicator of methylation status across multiple copies of a locus. This is of particular use when it is desirable to compare the methylation status of a sequence in a sample to a threshold or reference value.

As used herein, "methylation frequency" or "methylation percent (%)" refer to the number of instances in which a molecule or locus is methylated relative to the number of instances the molecule or locus is unmethylated.

As such, the methylation state describes the state of methylation of a nucleic acid (*e.g.*, a genomic sequence). In addition, the methylation state refers to the characteristics of a nucleic acid segment at a particular genomic locus relevant to methylation. Such characteristics include, but are not limited to, whether any of the cytosine (C) residues within this DNA sequence are methylated, the location of methylated C residue(s), the frequency or percentage of methylated C throughout any particular region of a nucleic acid, and allelic differences in methylation due to, *e.g.*, difference in the origin of the alleles. The terms "methylation state", "methylation profile", and "methylation status" also refer to the relative concentration, absolute concentration, or pattern of methylated C or unmethylated C throughout any particular region of a nucleic acid in a biological sample. For example, if the cytosine (C) residue(s) within a nucleic acid sequence are methylated it may be referred to as "hypermethylated" or having "increased methylation", whereas if the cytosine (C) residue(s) within a DNA sequence are not methylated it may be referred to as "hypomethylated" or having "decreased methylation". Likewise, if the cytosine (C) residue(s) within a nucleic acid sequence are methylated as compared to another nucleic acid sequence (*e.g.*, from a different region or from a different individual, etc.) that sequence is considered hypermethylated or having increased methylation compared to the other nucleic acid sequence. Alternatively, if the cytosine (C) residue(s) within a DNA sequence are not methylated as compared to another nucleic acid sequence (*e.g.*, from a different region or from a different individual, etc.) that sequence is considered hypomethylated or having decreased methylation compared to the other nucleic acid sequence. Additionally, the term "methylation pattern" as used herein refers to the collective sites of methylated and unmethylated nucleotides over a region

of a nucleic acid. Two nucleic acids may have the same or similar methylation frequency or methylation percent but have different methylation patterns when the number of methylated and unmethylated nucleotides is the same or similar throughout the region but the locations of methylated and unmethylated nucleotides are different. Sequences are said to be "differentially methylated" or as having a "difference in methylation" or having a "different methylation state" when they differ in the extent (*e.g.*, one has increased or decreased methylation relative to the other), frequency, or pattern of methylation. The term "differential methylation" refers to a difference in the level or pattern of nucleic acid methylation in a cancer positive sample as compared with the level or pattern of nucleic acid methylation in a cancer negative sample. It may also refer to the difference in levels or patterns between patients that have recurrence of cancer after surgery versus patients who not have recurrence. Differential methylation and specific levels or patterns of DNA methylation are prognostic and predictive biomarkers, *e.g.*, once the correct cut-off or predictive characteristics have been defined.

Methylation state frequency can be used to describe a population of individuals or a sample from a single individual. For example, a nucleotide locus having a methylation state frequency of 50% is methylated in 50% of instances and unmethylated in 50% of instances. Such a frequency can be used, for example, to describe the degree to which a nucleotide locus or nucleic acid region is methylated in a population of individuals or a collection of nucleic acids. Thus, when methylation in a first population or pool of nucleic acid molecules is different from methylation in a second population or pool of nucleic acid molecules, the methylation state frequency of the first population or pool will be different from the methylation state frequency of the second population or pool. Such a frequency also can be used, for example, to describe the degree to which a nucleotide locus or nucleic acid region is methylated in a single individual. For example, such a frequency can be used to describe the degree to which a group of cells from a tissue sample are methylated or unmethylated at a nucleotide locus or nucleic acid region.

As used herein a "nucleotide locus" refers to the location of a nucleotide in a nucleic acid molecule. A nucleotide locus of a methylated nucleotide refers to the location of a methylated nucleotide in a nucleic acid molecule.

Typically, methylation of human DNA occurs on a dinucleotide sequence including an adjacent guanine and cytosine where the cytosine is located 5' of the guanine (also termed CpG dinucleotide sequences). Most cytosines within the CpG dinucleotides are methylated in

the human genome, however some remain unmethylated in specific CpG dinucleotide rich genomic regions, known as CpG islands (see, *e.g.*, Antequera, *et al.* (1990) *Cell* 62: 503–514).

As used herein, a "CpG island" refers to a G:C-rich region of genomic DNA containing an increased number of CpG dinucleotides relative to total genomic DNA. A CpG island can be at least 100, 200, or more base pairs in length, where the G:C content of the region is at least 50% and the ratio of observed CpG frequency over expected frequency is 0.6; in some instances, a CpG island can be at least 500 base pairs in length, where the G:C content of the region is at least 55%) and the ratio of observed CpG frequency over expected frequency is 0.65. The observed CpG frequency over expected frequency can be calculated according to the method provided in Gardiner-Garden et al (1987) *J. Mol. Biol.* 196: 261–281. For example, the observed CpG frequency over expected frequency can be calculated according to the formula R = (A × B) / (C × D), where R is the ratio of observed CpG frequency over expected frequency, A is the number of CpG dinucleotides in an analyzed sequence, B is the total number of nucleotides in the analyzed sequence, C is the total number of C nucleotides in the analyzed sequence, and D is the total number of G nucleotides in the analyzed sequence. Methylation state is typically determined in CpG islands, *e.g.*, at promoter regions. It will be appreciated though that other sequences in the human genome are prone to DNA methylation such as CpA and CpT (see Ramsahoye (2000) *Proc. Natl. Acad. Sci. USA* 97: 5237–5242; Salmon and Kaye (1970) *Biochim. Biophys. Acta.* 204: 340–351; Grafstrom (1985) *Nucleic Acids Res.* 13: 2827–2842; Nyce (1986) *Nucleic Acids Res.* 14: 4353–4367; Woodcock (1987) *Biochem. Biophys. Res. Commun.* 145: 888-894).

As used herein, a "methylation-specific reagent" refers to a reagent that modifies a nucleotide of the nucleic acid molecule as a function of the methylation state of the nucleic acid molecule, or a methylation-specific reagent, refers to a compound or composition or other agent that can change the nucleotide sequence of a nucleic acid molecule in a manner that reflects the methylation state of the nucleic acid molecule. Methods of treating a nucleic acid molecule with such a reagent can include contacting the nucleic acid molecule with the reagent, coupled with additional steps, if desired, to accomplish the desired change of nucleotide sequence. Such methods can be applied in a manner in which unmethylated nucleotides (*e.g.*, each unmethylated cytosine) is modified to a different nucleotide. For example, in some embodiments, such a reagent can deaminate unmethylated cytosine nucleotides to produce deoxy uracil residues. An exemplary reagent is a bisulfite reagent.

The term "bisulfite reagent" refers to a reagent comprising bisulfite, disulfite, hydrogen sulfite, or combinations thereof, useful as disclosed herein to distinguish between methylated and unmethylated CpG dinucleotide sequences. Methods of said treatment are known in the art (*e.g.*, PCT/EP2004/011715 and WO 2013/116375, each of which is incorporated by reference in its entirety). In some embodiments, bisulfite treatment is conducted in the presence of denaturing solvents such as but not limited to n-alkyleneglycol or diethylene glycol dimethyl ether (DME), or in the presence of dioxane or dioxane derivatives. In some embodiments the denaturing solvents are used in concentrations between 1% and 35% (v/v). In some embodiments, the bisulfite reaction is carried out in the presence of scavengers such as but not limited to chromane derivatives, *e.g.*, 6-hydroxy-2,5,7,8,-tetramethylchromane 2-carboxylic acid or trihydroxybenzone acid and derivatives thereof, *e.g.*, Gallic acid (see: PCT/EP2004/011715, which is incorporated by reference in its entirety). In certain preferred embodiments, the bisulfite reaction comprises treatment with ammonium hydrogen sulfite, *e.g.*, as described in WO 2013/116375.

A change in the nucleic acid nucleotide sequence by a methylation –specific reagent can also result in a nucleic acid molecule in which each methylated nucleotide is modified to a different nucleotide.

The term "methylation assay" refers to any assay for determining the methylation state of one or more CpG dinucleotide sequences within a sequence of a nucleic acid.

As used herein, the "sensitivity" of a given marker (or set of markers used together) refers to the percentage of samples that report a DNA methylation value above a threshold value that distinguishes between neoplastic and non-neoplastic samples. In some embodiments, a positive is defined as a histology-confirmed neoplasia that reports a DNA methylation value above a threshold value (*e.g.*, the range associated with disease), and a false negative is defined as a histology-confirmed neoplasia that reports a DNA methylation value below the threshold value (*e.g.*, the range associated with no disease). The value of sensitivity, therefore, reflects the probability that a DNA methylation measurement for a given marker obtained from a known diseased sample will be in the range of disease-associated measurements. As defined here, the clinical relevance of the calculated sensitivity value represents an estimation of the probability that a given marker would detect the presence of a clinical condition when applied to a subject with that condition.

As used herein, the "specificity" of a given marker (or set of markers used together) refers to the percentage of non-neoplastic samples that report a DNA methylation value below a threshold value that distinguishes between neoplastic and non-neoplastic samples. In some embodiments, a negative is defined as a histology-confirmed non-neoplastic sample that reports a DNA methylation value below the threshold value (*e.g.*, the range associated with no disease) and a false positive is defined as a histology-confirmed non-neoplastic sample that reports a DNA methylation value above the threshold value (*e.g.*, the range associated with disease). The value of specificity, therefore, reflects the probability that a DNA methylation measurement for a given marker obtained from a known non-neoplastic sample will be in the range of non-disease associated measurements. As defined here, the clinical relevance of the calculated specificity value represents an estimation of the probability that a given marker would detect the absence of a clinical condition when applied to a patient without that condition.

As used herein, a "selected nucleotide" refers to one nucleotide of the four typically occurring nucleotides in a nucleic acid molecule (C, G, T, and A for DNA and C, G, U, and A for RNA), and can include methylated derivatives of the typically occurring nucleotides (*e.g.*, when C is the selected nucleotide, both methylated and unmethylated C are included within the meaning of a selected nucleotide), whereas a methylated selected nucleotide refers specifically to a nucleotide that is typically methylated and an unmethylated selected nucleotides refers specifically to a nucleotide that typically occurs in unmethylated form.

The term "methylation-specific restriction enzyme" refers to a restriction enzyme that selectively digests a nucleic acid dependent on the methylation state of its recognition site. In the case of a restriction enzyme that specifically cuts if the recognition site is not methylated or is hemi-methylated (a methylation-sensitive enzyme), the cut will not take place (or will take place with a significantly reduced efficiency) if the recognition site is methylated on one or both strands. In the case of a restriction enzyme that specifically cuts only if the recognition site is methylated (a methylation-dependent enzyme), the cut will not take place (or will take place with a significantly reduced efficiency) if the recognition site is not methylated. Preferred are methylation-specific restriction enzymes, the recognition sequence of which contains a CG dinucleotide (for instance a recognition sequence such as CGCG or CCCGGG). Further preferred for some embodiments are restriction enzymes that do not cut if the cytosine in this dinucleotide is methylated at the carbon atom C5.

The term "primer" refers to an oligonucleotide, whether occurring naturally as, *e.g.*, a nucleic acid fragment from a restriction digest, or produced synthetically, that is capable of acting as a point of initiation of synthesis when placed under conditions in which synthesis of a primer extension product that is complementary to a nucleic acid template strand is induced, (*e.g.*, in the presence of nucleotides and an inducing agent such as a DNA polymerase, and at a suitable temperature and pH). The primer is preferably single stranded for maximum efficiency in amplification, but may alternatively be double stranded. If double stranded, the primer is first treated to separate its strands before being used to prepare extension products. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the inducing agent. The exact lengths of the primers will depend on many factors, including temperature, source of primer, and the use of the method.

The term "probe" refers to an oligonucleotide (*e.g.*, a sequence of nucleotides), whether occurring naturally as in a purified restriction digest or produced synthetically, recombinantly, or by PCR amplification, that is capable of hybridizing to another oligonucleotide of interest. A probe may be single-stranded or double-stranded. Probes are useful in the detection, identification, and isolation of particular gene sequences (*e.g.*, a "capture probe"). It is contemplated that any probe used in the present invention may, in some embodiments, be labeled with any "reporter molecule," so that is detectable in any detection system, including, but not limited to enzyme (*e.g.*, ELISA, as well as enzyme-based histochemical assays), fluorescent, radioactive, and luminescent systems. It is not intended that the present invention be limited to any particular detection system or label.

The term "target," as used herein refers to a nucleic acid sought to be sorted out from other nucleic acids, *e.g.*, by probe binding, amplification, isolation, capture, *etc.* For example, when used in reference to the polymerase chain reaction, "target" refers to the region of nucleic acid bounded by the primers used for polymerase chain reaction, while when used in an assay in which target DNA is not amplified, *e.g.*, in some embodiments of an invasive cleavage assay, a target comprises the site at which a probe and invasive oligonucleotides (*e.g.*, INVADER oligonucleotide) bind to form an invasive cleavage structure, such that the presence of the target nucleic acid can be detected. A "segment" is defined as a region of nucleic acid within the target sequence. As used in reference to a double-stranded nucleic acid, the term "target" is not limited to a particular strand of the duplexed target, *e.g.*, a

coding strand, but may be used in reference to either or both strands of, for example, a double-stranded gene or reference DNA.

The term “marker”, as used herein, refers to a substance (*e.g.*, a nucleic acid, or a region of a nucleic acid, or a protein) that may be used to distinguish non-normal cells (*e.g.*, cancer cells) from normal cells (non-cancerous cells), *e.g.*, based on presence, absence, or status (*e.g.*, methylation state) of the marker substance. As used herein “normal” methylation of a marker refers to a degree of methylation typically found in normal cells, *e.g.*, in non-cancerous cells.

The term "neoplasm" as used herein refers to any new and abnormal growth of tissue. Thus, a neoplasm can be a premalignant neoplasm or a malignant neoplasm.

The term "neoplasm-specific marker," as used herein, refers to any biological material or element that can be used to indicate the presence of a neoplasm. Examples of biological materials include, without limitation, nucleic acids, polypeptides, carbohydrates, fatty acids, cellular components (*e.g.*, cell membranes and mitochondria), and whole cells. In some instances, markers are particular nucleic acid regions (*e.g.*, genes, intragenic regions, specific loci, etc.). Regions of nucleic acid that are markers may be referred to, *e.g.*, as "marker genes," "marker regions," "marker sequences," "marker loci," etc.

The term “sample” is used in its broadest sense. In one sense it can refer to an animal cell or tissue. In another sense, it refers to a specimen or culture obtained from any source, as well as biological and environmental samples. Biological samples may be obtained from plants or animals (including humans) and encompass fluids, solids, tissues, and gases. Environmental samples include environmental material such as surface matter, soil, water, and industrial samples. These examples are not to be construed as limiting the sample types applicable to the present invention.

As used herein, the terms “patient” or “subject” refer to organisms to be subject to various tests provided by the technology. The term “subject” includes animals, preferably mammals, including humans. In a preferred embodiment, the subject is a primate. In an even more preferred embodiment, the subject is a human. Further with respect to diagnostic methods, a preferred subject is a vertebrate subject. A preferred vertebrate is warm-blooded; a preferred warm-blooded vertebrate is a mammal. A preferred mammal is most preferably a human. As used herein, the term “subject' includes both human and animal subjects. Thus, veterinary therapeutic uses are provided herein. As such, the present technology provides for

the diagnosis of mammals such as humans, as well as those mammals of importance due to being endangered, such as Siberian tigers; of economic importance, such as animals raised on farms for consumption by humans; and/or animals of social importance to humans, such as animals kept as pets or in zoos. Examples of such animals include but are not limited to: carnivores such as cats and dogs; swine, including pigs, hogs, and wild boars; ruminants and/or ungulates such as cattle, oxen, sheep, giraffes, deer, goats, bison, and camels; pinnipeds; and horses. Thus, also provided is the diagnosis and treatment of livestock, including, but not limited to, domesticated swine, ruminants, ungulates, horses (including race horses), and the like. The presently-disclosed subject matter further includes a system for diagnosing a lung cancer in a subject. The system can be provided, for example, as a commercial kit that can be used to screen for a risk of lung cancer or diagnose a lung cancer in a subject from whom a biological sample has been collected. An exemplary system provided in accordance with the present technology includes assessing the methylation state of a marker described herein.

The term "amplifying" or "amplification" in the context of nucleic acids refers to the production of multiple copies of a polynucleotide, or a portion of the polynucleotide, typically starting from a small amount of the polynucleotide (*e.g.*, a single polynucleotide molecule), where the amplification products or amplicons are generally detectable. Amplification of polynucleotides encompasses a variety of chemical and enzymatic processes. The generation of multiple DNA copies from one or a few copies of a target or template DNA molecule during a polymerase chain reaction (PCR) or a ligase chain reaction (LCR; see, *e.g.*, U.S. Patent No. 5,494,810; herein incorporated by reference in its entirety) are forms of amplification. Additional types of amplification include, but are not limited to, allele-specific PCR (see, *e.g.*, U.S. Patent No. 5,639,611; herein incorporated by reference in its entirety), assembly PCR (see, *e.g.*, U.S. Patent No. 5,965,408; herein incorporated by reference in its entirety), helicase-dependent amplification (see, *e.g.*, U.S. Patent No. 7,662,594; herein incorporated by reference in its entirety), hot-start PCR (see, *e.g.*, U.S. Patent Nos. 5,773,258 and 5,338,671; each herein incorporated by reference in their entireties), intersequence-specific PCR, inverse PCR (see, *e.g.*, Triglia, *et al.*(1988) Nucleic Acids Res., 16:8186; herein incorporated by reference in its entirety), ligation-mediated PCR (see, *e.g.*, Guilfoyle, R. *et al.*, Nucleic Acids Research, 25:1854-1858 (1997); U.S. Patent No. 5,508,169; each of which are herein incorporated by reference in their entireties), methylation-specific PCR (see, *e.g.*, Herman, *et al.*, (1996) PNAS 93(13) 9821-9826; herein

incorporated by reference in its entirety), miniprimer PCR, multiplex ligation-dependent probe amplification (see, *e.g.*, Schouten, *et al.*, (2002) Nucleic Acids Research 30(12): e57; herein incorporated by reference in its entirety), multiplex PCR (see, *e.g.*, Chamberlain, *et al.*, (1988) Nucleic Acids Research 16(23) 11141-11156; Ballabio, *et al.*, (1990) Human Genetics 84(6) 571-573; Hayden, *et al.*, (2008) BMC Genetics 9:80; each of which are herein incorporated by reference in their entireties), nested PCR, overlap-extension PCR (see, *e.g.*, Higuchi, *et al.*, (1988) Nucleic Acids Research 16(15) 7351-7367; herein incorporated by reference in its entirety), real time PCR (see, *e.g.*, Higuchi, *et al.*, (1992) Biotechnology 10:413-417; Higuchi, *et al.*, (1993) Biotechnology 11:1026-1030; each of which are herein incorporated by reference in their entireties), reverse transcription PCR (see, *e.g.*, Bustin, S.A. (2000) J. Molecular Endocrinology 25:169-193; herein incorporated by reference in its entirety), solid phase PCR, thermal asymmetric interlaced PCR, and Touchdown PCR (see, *e.g.*, Don, *et al.*, Nucleic Acids Research (1991) 19(14) 4008; Roux, K. (1994) Biotechniques 16(5) 812-814; Hecker, *et al.*, (1996) Biotechniques 20(3) 478-485; each of which are herein incorporated by reference in their entireties). Polynucleotide amplification also can be accomplished using digital PCR (see, *e.g.*, Kalinina, *et al.*, Nucleic Acids Research. 25; 1999-2004, (1997); Vogelstein and Kinzler, Proc Natl Acad Sci USA. 96; 9236-41, (1999); International Patent Publication No. WO05023091A2; US Patent Application Publication No. 20070202525; each of which are incorporated herein by reference in their entireties).

The term "polymerase chain reaction" ("PCR") refers to the method of K.B. Mullis U.S. Patent Nos. 4,683,195, 4,683,202, and 4,965,188, that describe a method for increasing the concentration of a segment of a target sequence in a mixture of genomic or other DNA or RNA, without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the DNA mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a DNA polymerase. The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (*i.e.*, denaturation, annealing and extension constitute one "cycle"; there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the

desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" ("PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified" and are "PCR products" or "amplicons." Those of skill in the art will understand the term "PCR" encompasses many variants of the originally described method using, *e.g.*, real time PCR, nested PCR, reverse transcription PCR (RT-PCR), single primer and arbitrarily primed PCR, *etc.*

As used herein, the term "nucleic acid detection assay" refers to any method of determining the nucleotide composition of a nucleic acid of interest. Nucleic acid detection assay include but are not limited to, DNA sequencing methods, probe hybridization methods, structure specific cleavage assays (*e.g.*, the INVADER assay, (Hologic, Inc.) and are described, *e.g.*, in U.S. Patent Nos. 5,846,717, 5,985,557, 5,994,069, 6,001,567, 6,090,543, and 6,872,816; Lyamichev et al., Nat. Biotech., 17:292 (1999), Hall et al., PNAS, USA, 97:8272 (2000), and US Pat. No. 9,096,893, each of which is herein incorporated by reference in its entirety for all purposes); enzyme mismatch cleavage methods (*e.g.*, Variagenics, U.S. Pat. Nos. 6,110,684, 5,958,692, 5,851,770, herein incorporated by reference in their entireties); polymerase chain reaction (PCR), described above; branched hybridization methods (*e.g.*, Chiron, U.S. Pat. Nos. 5,849,481, 5,710,264, 5,124,246, and 5,624,802, herein incorporated by reference in their entireties); rolling circle replication (*e.g.*, U.S. Pat. Nos. 6,210,884, 6,183,960 and 6,235,502, herein incorporated by reference in their entireties); NASBA (*e.g.*, U.S. Pat. No. 5,409,818, herein incorporated by reference in its entirety); molecular beacon technology (*e.g.*, U.S. Pat. No. 6,150,097, herein incorporated by reference in its entirety); E-sensor technology (Motorola, U.S. Pat. Nos. 6,248,229, 6,221,583, 6,013,170, and 6,063,573, herein incorporated by reference in their entireties); cycling probe technology (*e.g.*, U.S. Pat. Nos. 5,403,711, 5,011,769, and 5,660,988, herein incorporated by reference in their entireties); Dade Behring signal amplification methods (*e.g.*, U.S. Pat. Nos. 6,121,001, 6,110,677, 5,914,230, 5,882,867, and 5,792,614, herein incorporated by reference in their entireties); ligase chain reaction (*e.g.*, Baranay Proc. Natl. Acad. Sci USA 88, 189-93 (1991)); and sandwich hybridization methods (*e.g.*, U.S. Pat. No. 5,288,609, herein incorporated by reference in its entirety).

In some embodiments, target nucleic acid is amplified (*e.g.*, by PCR) and amplified nucleic acid is detected simultaneously using an invasive cleavage assay. Assays configured for performing a detection assay (*e.g.*, invasive cleavage assay) in combination with an amplification assay are described in U.S. Pat. No. 9,096,893, incorporated herein by reference in its entirety for all purposes. Additional amplification plus invasive cleavage detection configurations, termed the QuARTS method, are described in, *e.g.*, in U.S. Pat. Nos. 8,361,720; 8,715,937; 8,916,344; 9,212,392, and U.S. Pat. Appl. No. 15/841,006 each of which is incorporated herein by reference for all purposes. The term "invasive cleavage structure" as used herein refers to a cleavage structure comprising i) a target nucleic acid, ii) an upstream nucleic acid (*e.g.*, an invasive or "INVADER" oligonucleotide), and iii) a downstream nucleic acid (*e.g.*, a probe), where the upstream and downstream nucleic acids anneal to contiguous regions of the target nucleic acid, and where an overlap forms between the a 3′ portion of the upstream nucleic acid and duplex formed between the downstream nucleic acid and the target nucleic acid. An overlap occurs where one or more bases from the upstream and downstream nucleic acids occupy the same position with respect to a target nucleic acid base, whether or not the overlapping base(s) of the upstream nucleic acid are complementary with the target nucleic acid, and whether or not those bases are natural bases or non-natural bases. In some embodiments, the 3′ portion of the upstream nucleic acid that overlaps with the downstream duplex is a non-base chemical moiety such as an aromatic ring structure, *e.g.*, as disclosed, for example, in U.S. Pat. No. 6,090,543, incorporated herein by reference in its entirety. In some embodiments, one or more of the nucleic acids may be attached to each other, *e.g.*, through a covalent linkage such as nucleic acid stem-loop, or through a non-nucleic acid chemical linkage (*e.g.*, a multi-carbon chain). As used herein, the term "flap endonuclease assay" includes "INVADER" invasive cleavage assays and QuARTS assays, as described above.

The term "probe oligonucleotide" or "flap oligonucleotide" when used in reference to flap assay, refers to an oligonucleotide that interacts with a target nucleic acid to form a cleavage structure in the presence of an invasive oligonucleotide.

The term "invasive oligonucleotide" refers to an oligonucleotide that hybridizes to a target nucleic acid at a location adjacent to the region of hybridization between a probe and the target nucleic acid, wherein the 3' end of the invasive oligonucleotide comprises a portion (*e.g.*, a chemical moiety, or one or more nucleotides) that overlaps with the region of hybridization between the probe and target. The 3' terminal nucleotide of the invasive

oligonucleotide may or may not base pair a nucleotide in the target. In some embodiments, the invasive oligonucleotide contains sequences at its 3' end that are substantially the same as sequences located at the 5' end of a portion of the probe oligonucleotide that anneals to the target strand.

The term "flap endonuclease" or "FEN," as used herein, refers to a class of nucleolytic enzymes, typically 5' nucleases, that act as structure-specific endonucleases on DNA structures with a duplex containing a single stranded 5' overhang, or flap, on one of the strands that is displaced by another strand of nucleic acid (*e.g.*, such that there are overlapping nucleotides at the junction between the single and double-stranded DNA). FENs catalyze hydrolytic cleavage of the phosphodiester bond at the junction of single and double stranded DNA, releasing the overhang, or the flap. Flap endonucleases are reviewed by Ceska and Savers (Trends Biochem. Sci. 1998 23:331-336) and Liu et al (Annu. Rev. Biochem. 2004 73: 589-615; herein incorporated by reference in its entirety). FENs may be individual enzymes, multi-subunit enzymes, or may exist as an activity of another enzyme or protein complex (*e.g.*, a DNA polymerase).

A flap endonuclease may be thermostable. For example, FEN-1 flap endonuclease from archival thermophiles organisms are typical thermostable. As used herein, the term "FEN-1" refers to a non-polymerase flap endonuclease from a eukaryote or archaeal organism. See, *e.g.*, WO 02/070755, and Kaiser M.W., *et al.* (1999) J. Biol. Chem., 274:21387, which are incorporated by reference herein in their entireties for all purposes.

As used herein, the term "cleaved flap" refers to a single-stranded oligonucleotide that is a cleavage product of a flap assay.

The term "cassette," when used in reference to a flap cleavage reaction, refers to an oligonucleotide or combination of oligonucleotides configured to generate a detectable signal in response to cleavage of a flap or probe oligonucleotide, *e.g.*, in a primary or first cleavage structure formed in a flap cleavage assay. In preferred embodiments, the cassette hybridizes to a non-target cleavage product produced by cleavage of a flap oligonucleotide to form a second overlapping cleavage structure, such that the cassette can then be cleaved by the same enzyme, *e.g.*, a FEN-1 endonuclease.

In some embodiments, the cassette is a single oligonucleotide comprising a hairpin portion (*i.e.*, a region wherein one portion of the cassette oligonucleotide hybridizes to a second portion of the same oligonucleotide under reaction conditions, to form a duplex). In other embodiments, a cassette comprises at least two oligonucleotides comprising complementary portions that can form a duplex under reaction conditions. In preferred

embodiments, the cassette comprises a label, *e.g.*, a fluorophore. In particularly preferred embodiments, a cassette comprises labeled moieties that produce a FRET effect.

As used herein, the term "FRET" refers to fluorescence resonance energy transfer, a process in which moieties (*e.g.*, fluorophores) transfer energy *e.g.*, among themselves, or, from a fluorophore to a non-fluorophore (*e.g.*, a quencher molecule). In some circumstances, FRET involves an excited donor fluorophore transferring energy to a lower-energy acceptor fluorophore via a short-range (*e.g.*, about 10 nm or less) dipole-dipole interaction. In other circumstances, FRET involves a loss of fluorescence energy from a donor and an increase in fluorescence in an acceptor fluorophore. In still other forms of FRET, energy can be exchanged from an excited donor fluorophore to a non-fluorescing molecule (*e.g.*, a "dark" quenching molecule). FRET is known to those of skill in the art and has been described (See, *e.g.*, Stryer *et al.*, 1978, Ann. Rev. Biochem., 47:819; Selvin, 1995, Methods Enzymol., 246:300; Orpana, 2004 Biomol Eng 21, 45-50; Olivier, 2005 Mutant Res 573, 103-110, each of which is incorporated herein by reference in its entirety).

In an exemplary flap detection assay, an invasive oligonucleotide and flap oligonucleotide are hybridized to a target nucleic acid to produce a first complex having an overlap as described above. An unpaired "flap" is included on the 5' end of the flap oligonucleotide. The first complex is a substrate for a flap endonuclease, *e.g.*, a FEN-1 endonuclease, which cleaves the flap oligonucleotide to release the 5' flap portion. In a secondary reaction, the released 5' flap product serves as an invasive oligonucleotide on a FRET cassette to again create the structure recognized by the flap endonuclease, such that the FRET cassette is cleaved. When the fluorophore and the quencher are separated by cleavage of the FRET cassette, a detectable fluorescent signal above background fluorescence is produced.

The term "real time" as used herein in reference to detection of nucleic acid amplification or signal amplification refers to the detection or measurement of the accumulation of products or signal in the reaction while the reaction is in progress, *e.g.*, during incubation or thermal cycling. Such detection or measurement may occur continuously, or it may occur at a plurality of discrete points during the progress of the amplification reaction, or it may be a combination. For example, in a polymerase chain reaction, detection (*e.g.*, of fluorescence) may occur continuously during all or part of thermal cycling, or it may occur transiently, at one or more points during one or more cycles. In some embodiments, real time detection of PCR or QuARTS reactions is accomplished by

determining a level of fluorescence at the same point (*e.g.*, a time point in the cycle, or temperature step in the cycle) in each of a plurality of cycles, or in every cycle. Real time detection of amplification may also be referred to as detection "during" the amplification reaction.

As used herein, the term "quantitative amplification data set" refers to the data obtained during quantitative amplification of the target sample, *e.g.*, target DNA. In the case of quantitative PCR or QuARTS assays, the quantitative amplification data set is a collection of fluorescence values obtained at during amplification, *e.g.*, during a plurality of, or all of the thermal cycles. Data for quantitative amplification is not limited to data collected at any particular point in a reaction, and fluorescence may be measured at a discrete point in each cycle or continuously throughout each cycle.

The abbreviations "Ct" and "Cp" as used herein in reference to data collected during real time PCR and PCR+INVADER assays refer to the cycle at which signal (*e.g.*, fluorescent signal) crosses a predetermined threshold value indicative of positive signal. Various methods have been used to calculate the threshold that is used as a determinant of signal verses concentration, and the value is generally expressed as either the "crossing threshold" (Ct) or the "crossing point" (Cp). Either Cp values or Ct values may be used in embodiments of the methods presented herein for analysis of real-time signal for the determination of the percentage of variant and/or non-variant constituents in an assay or sample.

As used herein, the term “kit” refers to any delivery system for delivering materials. In the context of reaction assays, such delivery systems include systems that allow for the storage, transport, or delivery of reaction reagents (*e.g.*, oligonucleotides, enzymes, etc. in the appropriate containers) and/or supporting materials (*e.g.*, buffers, written instructions for performing the assay etc.) from one location to another. For example, kits include one or more enclosures (*e.g.*, boxes) containing the relevant reaction reagents and/or supporting materials. As used herein, the term “fragmented kit” refers to delivery systems comprising two or more separate containers that each contains a subportion of the total kit components. The containers may be delivered to the intended recipient together or separately. For example, a first container may contain an enzyme for use in an assay, while a second container contains oligonucleotides.

The term “system” as used herein refers to a collection of articles for use for a particular purpose. In some embodiments, the articles comprise instructions for use, as information

supplied on *e.g.*, an article, on paper, or on recordable media (*e.g.*, DVD, CD, flash drive, *etc.*). In some embodiments, instructions direct a user to an online location, *e.g.*, a website.

As used herein, the term "information" refers to any collection of facts or data. In reference to information stored or processed using a computer system(s), including but not limited to internets, the term refers to any data stored in any format (*e.g.*, analog, digital, optical, *etc.*). As used herein, the term "information related to a subject" refers to facts or data pertaining to a subject (*e.g.*, a human, plant, or animal). The term "genomic information" refers to information pertaining to a genome including, but not limited to, nucleic acid sequences, genes, percentage methylation, allele frequencies, RNA expression levels, protein expression, phenotypes correlating to genotypes, *etc.* "Allele frequency information" refers to facts or data pertaining to allele frequencies, including, but not limited to, allele identities, statistical correlations between the presence of an allele and a characteristic of a subject (*e.g.*, a human subject), the presence or absence of an allele in an individual or population, the percentage likelihood of an allele being present in an individual having one or more particular characteristics, *etc.*

DESCRIPTION OF THE DRAWINGS

Figure 1 shows schematic diagrams of marker target regions in unconverted form and bisulfite-converted form. Flap assay primers and probes for detection of bisulfite-converted target DNA are shown.

Figures 2-5 provide tables comparing Reduced Representation Bisulfite Sequencing (RRBS) results for selecting markers associated with lung carcinomas as described in Example 2, with each row showing the mean values for the indicated marker region (identified by chromosome and start and stop positions). The ratio of mean methylation for each tissue type (normal (Norm), adenocarcinoma (Ad), large cell carcinoma (LC), small cell carcinoma(SC), squamous cell carcinoma (SQ) and undefined cancer (UND)) is compared to the mean methylation of buffy coat samples from normal subjects (WBC or BC)) is shown for each region, and genes and transcripts identified with each region are indicated.

Figure 2 provides a table comparing RRBS results for selecting markers associated with lung adenocarcinoma.

Figure 3 provides a table comparing RRBS results for selecting markers associated with lung large cell carcinoma.

Figure 4 provides a table comparing RRBS results for selecting markers associated with lung small cell carcinoma.

Figure 5 provides a table comparing RRBS results for selecting markers associated with lung squamous cell carcinoma.

Figure 6 provides a table of nucleic acid sequences of assay targets and detection oligonucleotides, with corresponding SEQ ID NOS. Target nucleic acids, in particular target DNAs (including bisulfite-converted DNAs) are shown for convenience as single strands but it is understood that embodiments of the technology encompass the complementary strands of the depicted sequences. For example, primers and flap oligonucleotides may be selected to hybridize to the targets as shown, or to strands that are complementary to the targets as shown.

Figure 7 provides a graph showing a 6-marker logistic fit of data from Example 3, using markers *SHOX2, SOBP, ZNF781, BTACT, CYP26C1, and DLX4.* The ROC curve analysis shows an area under the curve (AUC) of 0.973.

Figure 8 provides a graph showing a 6-marker logistic fit of data from Example 3, using markers *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2, and SKI.* The ROC curve analysis shows an area under the curve (AUC) of 0.97982.

Figure 9A-9I show graphs showing individual marker logistic fit of data from Example 6.

Figure 10 provides a graph showing a 6-marker logistic fit of data from Example 6, using markers *BARX1, FLJ45983, SOBP, HOPX, IFFO1,* and *ZNF781.*

DETAILED DESCRIPTION OF THE INVENTION

Provided herein is technology relating to selection of nucleic acid markers for use in assays for detection and quantification of DNA, *e.g.*, methylated DNA, and use of the markers in nucleic acid detection assays. In particular, the technology relates to use of methylation assays to detect lung cancer.

In this detailed description of the various embodiments, for purposes of explanation, numerous specific details are set forth to provide a thorough understanding of the embodiments disclosed. One skilled in the art will appreciate, however, that these various embodiments may be practiced with or without these specific details. In other instances, structures and devices are shown in block diagram form. Furthermore, one skilled in the art

can readily appreciate that the specific sequences in which methods are presented and performed are illustrative and it is contemplated that the sequences can be varied and still remain within the spirit and scope of the various embodiments disclosed herein.

In some embodiments, a marker is a region of 100 or fewer bases, the marker is a region of 500 or fewer bases, the marker is a region of 1000 or fewer bases, the marker is a region of 5000 or fewer bases, or, in some embodiments, the marker is one base. In some embodiments the marker is in a high CpG density promoter.

The technology is not limited by sample type. For example, in some embodiments the sample is a stool sample, a tissue sample, sputum, a blood sample (*e.g.*, plasma, serum, whole blood), an excretion, or a urine sample.

Furthermore, the technology is not limited in the method used to determine methylation state. In some embodiments the assaying comprises using methylation specific polymerase chain reaction, nucleic acid sequencing, mass spectrometry, methylation specific nuclease, mass-based separation, or target capture. In some embodiments, the assaying comprises use of a methylation specific oligonucleotide. In some embodiments, the technology uses massively parallel sequencing (*e.g.*, next-generation sequencing) to determine methylation state, *e.g.*, sequencing-by-synthesis, real-time (*e.g.*, single-molecule) sequencing, bead emulsion sequencing, nanopore sequencing, etc.

The technology provides reagents for detecting a differentially methylated region (DMR). In some embodiments, an oligonucleotide is provided, the oligonucleotide comprising a sequence complementary to a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably to a marker selected from the subset *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2, EMX1, CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9,*

B3GALT6, ZNF781, SP9, BARX1, and *SKI;* or a marker selected from any of the subsets of markers defining the group consisting of *ZNF781, BARX1,* and *EMX1*; the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI;* the group consisting of *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1;* the group consisting of *SHOX2, SOBP, ZNF781, BTACT, CYP26C1,* and *DLX4;* or the group consisting of *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI.*

Kit embodiments are provided, *e.g.*, a kit comprising a bisulfite reagent; and a control nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, and having a methylation state associated with a subject who does not have a cancer (*e.g.*, lung cancer). In some embodiments, kits comprise a bisulfite reagent and an oligonucleotide as described herein. In some embodiments, kits comprise a bisulfite reagent; and a control nucleic acid comprising a sequence from such a chromosomal region and having a methylation state associated with a subject who has lung cancer.

The technology is related to embodiments of compositions (*e.g.*, reaction mixtures). In some embodiments are provided a composition comprising a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any

of the subsets of markers as recited above, and a bisulfite reagent. Some embodiments provide a composition comprising a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, and an oligonucleotide as described herein. Some embodiments provide a composition comprising a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, and a methylation-specific restriction enzyme. Some embodiments provide a composition comprising a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX*, preferably from any of the subsets of markers as recited above, and a polymerase.

Additional related method embodiments are provided for screening for a neoplasm (*e.g.*, lung carcinoma) in a sample obtained from a subject, *e.g.*, a method comprising determining a methylation state of a marker in the sample comprising a base in a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, ; comparing the methylation state of the marker from the subject sample to a methylation state of the marker from a normal control sample from a subject who does not have lung cancer; and determining a confidence interval and/or a p value of the difference in the methylation state of the subject sample and the normal control sample. In some embodiments, the confidence interval is 90%, 95%, 97.5%, 98%, 99%, 99.5%, 99.9% or 99.99% and the p value is 0.1, 0.05, 0.025, 0.02, 0.01, 0.005, 0.001, or 0.0001. Some embodiments of methods provide steps of reacting a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, with a bisulfite reagent to produce a bisulfite-reacted nucleic acid; sequencing the bisulfite-reacted nucleic acid to provide a nucleotide sequence of the bisulfite-reacted nucleic acid; comparing the nucleotide sequence of the bisulfite-reacted nucleic acid with a nucleotide sequence of a nucleic acid comprising the chromosomal region from a subject who does not have lung cancer to identify differences in

the two sequences; and identifying the subject as having a neoplasm when a difference is present.

Systems for screening for lung cancer in a sample obtained from a subject are provided by the technology. Exemplary embodiments of systems include, *e.g.*, a system for screening for lung cancer in a sample obtained from a subject, the system comprising an analysis component configured to determine the methylation state of a sample, a software component configured to compare the methylation state of the sample with a control sample or a reference sample methylation state recorded in a database, and an alert component configured to alert a user of a cancer-associated methylation state. An alert is determined in some embodiments by a software component that receives the results from multiple assays (*e.g.*, determining the methylation states of multiple markers, *e.g.*, a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, and calculating a value or result to report based on the multiple results. Some embodiments provide a database of weighted parameters associated with each a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above, provided herein for use in calculating a value or result and/or an alert to report to a user (*e.g.*, such as a physician, nurse, clinician, etc.). In

some embodiments all results from multiple assays are reported and in some embodiments one or more results are used to provide a score, value, or result based on a composite of one or more results from multiple assays that is indicative of a lung cancer risk in a subject.

In some embodiments of systems, a sample comprises a nucleic acid comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above. In some embodiments the system further comprises a component for isolating a nucleic acid, a component for collecting a sample such as a component for collecting a stool sample. In some embodiments, the system comprises nucleic acid sequences comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above. In some embodiments the database comprises nucleic acid sequences from subjects who do not have lung cancer. Also provided are nucleic acids, *e.g.*, a set of nucleic acids, each nucleic acid having a sequence comprising a chromosomal region having an annotation selected from *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX*

chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1, and *HOPX*, preferably from any of the subsets of markers as recited above.

Related system embodiments comprise a set of nucleic acids as described, and a database of nucleic acid sequences associated with the set of nucleic acids. Some embodiments further comprise a bisulfite reagent. And, some embodiments further comprise a nucleic acid sequencer.

In certain embodiments, methods for characterizing a sample obtained from a human subject are provided, comprising a) obtaining a sample from a human subject; b) assaying a methylation state of one or more markers in the sample, wherein the marker comprises a base in a chromosomal region having an annotation selected from the following groups of markers: *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX,* preferably from any of the subsets of markers as recited above; and c) comparing the methylation state of the assayed marker to the methylation state of the marker assayed in a subject that does not have a neoplasm.

In some embodiments, the technology is related to assessing the presence of and methylation state of one or more of the markers identified herein in a biological sample. These markers comprise one or more differentially methylated regions (DMR) as discussed herein. Methylation state is assessed in embodiments of the technology. As such, the technology provided herein is not restricted in the method by which a gene's methylation state is measured. For example, in some embodiments the methylation state is measured by a genome scanning method. For example, one method involves restriction landmark genomic scanning (Kawai et al. (1994) *Mol. Cell. Biol.* **14**: 7421–7427) and another example involves

methylation-specific arbitrarily primed PCR (Gonzalgo et al. (1997) *Cancer Res.* **57**: 594–599). In some embodiments, changes in methylation patterns at specific CpG sites are monitored by digestion of genomic DNA with methylation-specific restriction enzymes, particularly methylation-sensitive enzymes, followed by Southern analysis of the regions of interest (digestion-Southern method). In some embodiments, analyzing changes in methylation patterns involves a process comprising digestion of genomic DNA with one or more methylation-specific restriction enzymes, and analyzing regions for cleavage or non-cleavage indicating the methylation status of analyzed regions. In some embodiments, analysis of the treated DNA comprises PCR amplification, with the amplification result indicating whether the DNA was or was not cleaved by the restriction enzyme. In some embodiments, one or more of the presence, absence, amount, size, and sequence of an amplification product produced is assessed to analyze the methylation status of a DNA of interest. See, *e.g.*, Melnikov, *et al.,* (2005) *Nucl. Acids Res*, 33(10):e93; Hua, *et al.,* (2011) *Exp. Mol. Pathol.* 91(1):455-60; and Singer-Sam et al. (1990) *Nucl. Acids Res.* **18**: 687. In addition, other techniques have been reported that utilize bisulfite treatment of DNA as a starting point for methylation analysis. These include methylation-specific PCR (MSP) (Herman et al. (1992) *Proc. Natl. Acad. Sci. USA* **93**: 9821–9826) and restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA (Sadri and Hornsby (1996) *Nucl. Acids Res.* **24**: 5058–5059; and Xiong and Laird (1997) *Nucl. Acids Res.* **25**: 2532–2534). PCR techniques have been developed for detection of gene mutations (Kuppuswamy et al. (1991) *Proc. Natl. Acad. Sci. USA* **88**: 1143–1147) and quantification of allelic-specific expression (Szabo and Mann (1995) *Genes Dev.* **9**: 3097–3108; and Singer-Sam et al. (1992) *PCR Methods Appl.* **1**: 160–163). Such techniques use internal primers, which anneal to a PCR-generated template and terminate immediately 5' of the single nucleotide to be assayed. Methods using a "quantitative Ms-SNuPE assay" as described in U.S. Pat. No. 7,037,650 are used in some embodiments.

Upon evaluating a methylation state, the methylation state is often expressed as the fraction or percentage of individual strands of DNA that is methylated at a particular site (*e.g.*, at a single nucleotide, at a particular region or locus, at a longer sequence of interest, *e.g.*, up to a ~100-bp, 200-bp, 500-bp, 1000-bp subsequence of a DNA or longer) relative to the total population of DNA in the sample comprising that particular site. Traditionally, the amount of the unmethylated nucleic acid is determined by PCR using calibrators. Then, a known amount of DNA is bisulfite treated and the resulting methylation-specific sequence is

determined using either a real-time PCR or other exponential amplification, *e.g.*, a QuARTS assay (*e.g.*, as provided by U.S. Pat. Nos. 8,361,720; 8,715,937; 8,916,344; and 9,212,392, and U.S. Pat. Appl. Ser No. 15/841,006).

For example, in some embodiments, methods comprise generating a standard curve for the unmethylated target by using external standards. The standard curve is constructed from at least two points and relates the real-time Ct value for unmethylated DNA to known quantitative standards. Then, a second standard curve for the methylated target is constructed from at least two points and external standards. This second standard curve relates the Ct for methylated DNA to known quantitative standards. Next, the test sample Ct values are determined for the methylated and unmethylated populations and the genomic equivalents of DNA are calculated from the standard curves produced by the first two steps. The percentage of methylation at the site of interest is calculated from the amounts of methylated DNAs relative to the total amount of DNAs in the population, *e.g.*, (number of methylated DNAs) / (the number of methylated DNAs + number of unmethylated DNAs) × 100.

Also provided herein are compositions and kits for practicing the methods. For example, in some embodiments, reagents (*e.g.*, primers, probes) specific for one or more markers are provided alone or in sets (*e.g.*, sets of primers pairs for amplifying a plurality of markers). Additional reagents for conducting a detection assay may also be provided (*e.g.*, enzymes, buffers, positive and negative controls for conducting QuARTS, PCR, sequencing, bisulfite, or other assays). In some embodiments, the kits containing one or more reagent necessary, sufficient, or useful for conducting a method are provided. Also provided are reactions mixtures containing the reagents. Further provided are master mix reagent sets containing a plurality of reagents that may be added to each other and/or to a test sample to complete a reaction mixture.

Methods for isolating DNA suitable for these assay technologies are known in the art. In particular, some embodiments comprise isolation of nucleic acids as described in U.S. Pat. Appl. Ser. No. 13/470,251 ("Isolation of Nucleic Acids"), incorporated herein by reference in its entirety.

Genomic DNA may be isolated by any means, including the use of commercially available kits. Briefly, wherein the DNA of interest is encapsulated by a cellular membrane the biological sample must be disrupted and lysed by enzymatic, chemical or mechanical means. The DNA solution may then be cleared of proteins and other contaminants, *e.g.*, by

digestion with proteinase K. The genomic DNA is then recovered from the solution. This may be carried out by means of a variety of methods including salting out, organic extraction, or binding of the DNA to a solid phase support. The choice of method will be affected by several factors including time, expense, and required quantity of DNA. All clinical sample types comprising neoplastic matter or pre-neoplastic matter are suitable for use in the present method, *e.g.*, cell lines, histological slides, biopsies, paraffin-embedded tissue, body fluids, stool, colonic effluent, urine, blood plasma, blood serum, whole blood, isolated blood cells, cells isolated from the blood, and combinations thereof.

The technology is not limited in the methods used to prepare the samples and provide a nucleic acid for testing. For example, in some embodiments, a DNA is isolated from a stool sample or from blood or from a plasma sample using direct gene capture, *e.g.*, as detailed in U.S. Pat. Appl. Ser. No. 61/485386 or by a related method.

The technology relates to the analysis of any sample that may be associated with lung cancer, or that may be examined to establish the absence of lung cancer. For example, in some embodiments the sample comprises a tissue and/or biological fluid obtained from a patient. In some embodiments, the sample comprises a secretion. In some embodiments, the sample comprises sputum, blood, serum, plasma, gastric secretions, lung tissue samples, lung cells or lung DNA recovered from stool. In some embodiments, the subject is human. Such samples can be obtained by any number of means known in the art, such as will be apparent to the skilled person.

I. Methylation assays to detect lung cancer

Candidate methylated DNA markers were identified by unbiased whole methylome sequencing of selected lung cancer case and lung control tissues. The top marker candidates were further evaluated in 255 independent patients with 119 controls, of which 37 were from benign nodules, and 136 cases inclusive of all lung cancer subtypes. DNA extracted from patient tissue samples was bisulfite treated and then candidate markers and β-actin (ACTB) as a normalizing gene were assayed by Quantitative Allele-Specific Real-time Target and Signal amplification (QuARTS amplification). QuARTS assay chemistry yields high discrimination for methylation marker selection and screening.

On receiver operator characteristics analyses of individual marker candidates, areas under the curve (AUCs) ranged from 0.512 to 0.941. At 100% specificity, a combined panel of 8 methylation markers (*SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.12.526,*

HOXB2, and *EMX1*) yielded a sensitivity of 98.5% across all subtypes of lung cancer. Furthermore, using the 8 markers panel, benign lung nodules yielded no false positives.

II. Methylation Detection Assays and Kits

The markers described herein find use in a variety of methylation detection assays. The most frequently used method for analyzing a nucleic acid for the presence of 5-methylcytosine is based upon the bisulfite method described by Frommer, et al. for the detection of 5-methylcytosines in DNA (Frommer et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 1827–31 explicitly incorporated herein by reference in its entirety for all purposes) or variations thereof. The bisulfite method of mapping 5-methylcytosines is based on the observation that cytosine, but not 5-methylcytosine, reacts with hydrogen sulfite ion (also known as bisulfite). The reaction is usually performed according to the following steps: first, cytosine reacts with hydrogen sulfite to form a sulfonated cytosine. Next, spontaneous deamination of the sulfonated reaction intermediate results in a sulfonated uracil. Finally, the sulfonated uracil is desulfonated under alkaline conditions to form uracil. Detection is possible because uracil base pairs with adenine (thus behaving like thymine), whereas 5-methylcytosine base pairs with guanine (thus behaving like cytosine). This makes the discrimination of methylated cytosines from non-methylated cytosines possible by, *e.g.*, bisulfite genomic sequencing (Grigg G, & Clark S, Bioessays (1994) 16: 431–36; Grigg G, DNA Seq. (1996) 6: 189–98),methylation-specific PCR (MSP) as is disclosed, *e.g.*, in U.S. Patent No. 5,786,146, or using an assay comprising sequence-specific probe cleavage, *e.g.*, a QuARTS flap endonuclease assay (see, *e.g.*, Zou et al. (2010) "Sensitive quantification of methylated markers with a novel methylation specific technology" *Clin Chem* **56**: A199; and in U.S. Pat. Nos. 8,361,720; 8,715,937; 8,916,344; and 9,212,392.

Some conventional technologies are related to methods comprising enclosing the DNA to be analyzed in an agarose matrix, thereby preventing the diffusion and renaturation of the DNA (bisulfite only reacts with single-stranded DNA), and replacing precipitation and purification steps with a fast dialysis (Olek A, et al. (1996) "A modified and improved method for bisulfite based cytosine methylation analysis" *Nucleic Acids Res.* **24**: 5064-6). It is thus possible to analyze individual cells for methylation status, illustrating the utility and sensitivity of the method. An overview of conventional methods for detecting 5-methylcytosine is provided by Rein, T., et al. (1998) *Nucleic Acids Res.* **26**: 2255.

The bisulfite technique typically involves amplifying short, specific fragments of a known nucleic acid subsequent to a bisulfite treatment, then either assaying the product by sequencing (Olek & Walter (1997) *Nat. Genet.* **17**: 275–6) or a primer extension reaction (Gonzalgo & Jones (1997) *Nucleic Acids Res.* **25**: 2529–31; WO 95/00669; U.S. Pat. No. 6,251,594) to analyze individual cytosine positions. Some methods use enzymatic digestion (Xiong & Laird (1997) *Nucleic Acids Res.* **25**: 2532–4). Detection by hybridization has also been described in the art (Olek et al., WO 99/28498). Additionally, use of the bisulfite technique for methylation detection with respect to individual genes has been described (Grigg & Clark (1994) *Bioessays* **16**: 431–6; Zeschnigk et al. (1997) *Hum Mol Genet.* **6**: 387–95; Feil et al. (1994) *Nucleic Acids Res.* 22: 695; Martin et al. (1995) *Gene* **157**: 261–4; WO 9746705; WO 9515373).

Various methylation assay procedures can be used in conjunction with bisulfite treatment according to the present technology. These assays allow for determination of the methylation state of one or a plurality of CpG dinucleotides (*e.g.*, CpG islands) within a nucleic acid sequence. Such assays involve, among other techniques, sequencing of bisulfite-treated nucleic acid, PCR (for sequence-specific amplification), Southern blot analysis, and use of methylation-specific restriction enzymes, *e.g.*, methylation-sensitive or methylation-dependent enzymes.

For example, genomic sequencing has been simplified for analysis of methylation patterns and 5-methylcytosine distributions by using bisulfite treatment (Frommer et al. (1992) *Proc. Natl. Acad. Sci. USA* **89**: 1827–1831). Additionally, restriction enzyme digestion of PCR products amplified from bisulfite-converted DNA finds use in assessing methylation state, *e.g.*, as described by Sadri & Hornsby (1997) *Nucl. Acids Res.* **24**: 5058–5059 or as embodied in the method known as COBRA (Combined Bisulfite Restriction Analysis) (Xiong & Laird (1997) *Nucleic Acids Res.* **25**: 2532–2534).

COBRA™ analysis is a quantitative methylation assay useful for determining DNA methylation levels at specific loci in small amounts of genomic DNA (Xiong & Laird, Nucleic Acids Res. 25:2532-2534, 1997). Briefly, restriction enzyme digestion is used to reveal methylation-dependent sequence differences in PCR products of sodium bisulfite-treated DNA. Methylation-dependent sequence differences are first introduced into the genomic DNA by standard bisulfite treatment according to the procedure described by Frommer et al. (Proc. Natl. Acad. Sci. USA 89:1827-1831, 1992). PCR amplification of the bisulfite converted DNA is then performed using primers specific for the CpG islands of

interest, followed by restriction endonuclease digestion, gel electrophoresis, and detection using specific, labeled hybridization probes. Methylation levels in the original DNA sample are represented by the relative amounts of digested and undigested PCR product in a linearly quantitative fashion across a wide spectrum of DNA methylation levels. In addition, this technique can be reliably applied to DNA obtained from microdissected paraffin-embedded tissue samples.

Typical reagents (*e.g.*, as might be found in a typical COBRA™-based kit) for COBRA™ analysis may include, but are not limited to: PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, *etc.*); restriction enzyme and appropriate buffer; gene-hybridization oligonucleotide; control hybridization oligonucleotide; kinase labeling kit for oligonucleotide probe; and labeled nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kits (*e.g.*, precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Assays such as "MethyLight™" (a fluorescence-based real-time PCR technique) (Eads et al., Cancer Res. 59:2302-2306, 1999), Ms-SNuPE™ (Methylation-sensitive Single Nucleotide Primer Extension) reactions (Gonzalgo & Jones, Nucleic Acids Res. 25:2529-2531, 1997), methylation-specific PCR ("MSP"; Herman et al., Proc. Natl. Acad. Sci. USA 93:9821-9826, 1996; U.S. Pat. No. 5,786,146), and methylated CpG island amplification ("MCA"; Toyota et al., Cancer Res. 59:2307-12, 1999) are used alone or in combination with one or more of these methods.

The "HeavyMethyl™" assay, technique is a quantitative method for assessing methylation differences based on methylation-specific amplification of bisulfite-treated DNA. Methylation-specific blocking probes ("blockers") covering CpG positions between, or covered by, the amplification primers enable methylation-specific selective amplification of a nucleic acid sample.

The term "HeavyMethyl™ MethyLight™" assay refers to a HeavyMethyl™ MethyLight™ assay, which is a variation of the MethyLight™ assay, wherein the MethyLight™ assay is combined with methylation specific blocking probes covering CpG positions between the amplification primers. The HeavyMethyl™ assay may also be used in combination with methylation specific amplification primers.

Typical reagents (*e.g.*, as might be found in a typical MethyLight™-based kit) for HeavyMethyl™ analysis may include, but are not limited to: PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, or bisulfite treated DNA sequence or CpG island, *etc.*); blocking oligonucleotides; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

MSP (methylation-specific PCR) allows for assessing the methylation status of virtually any group of CpG sites within a CpG island, independent of the use of methylation-specific restriction enzymes (Herman et al. Proc. Natl. Acad. Sci. USA 93:9821-9826, 1996; U.S. Pat. No. 5,786,146). Briefly, DNA is modified by sodium bisulfite, which converts unmethylated, but not methylated cytosines, to uracil, and the products are subsequently amplified with primers specific for methylated versus unmethylated DNA. MSP requires only small quantities of DNA, is sensitive to 0.1% methylated alleles of a given CpG island locus, and can be performed on DNA extracted from paraffin-embedded samples. Typical reagents (*e.g.*, as might be found in a typical MSP-based kit) for MSP analysis may include, but are not limited to: methylated and unmethylated PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, *etc.*); optimized PCR buffers and deoxynucleotides, and specific probes.

The MethyLight™ assay is a high-throughput quantitative methylation assay that utilizes fluorescence-based real-time PCR (*e.g.*, TaqMan®) that requires no further manipulations after the PCR step (Eads et al., Cancer Res. 59:2302-2306, 1999). Briefly, the MethyLight™ process begins with a mixed sample of genomic DNA that is converted, in a sodium bisulfite reaction, to a mixed pool of methylation-dependent sequence differences according to standard procedures (the bisulfite process converts unmethylated cytosine residues to uracil). Fluorescence-based PCR is then performed in a "biased" reaction, *e.g.*, with PCR primers that overlap known CpG dinucleotides. Sequence discrimination occurs both at the level of the amplification process and at the level of the fluorescence detection process.

The MethyLight™ assay is used as a quantitative test for methylation patterns in a nucleic acid, *e.g.*, a genomic DNA sample, wherein sequence discrimination occurs at the level of probe hybridization. In a quantitative version, the PCR reaction provides for a methylation specific amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe, overlie any CpG

dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing the biased PCR pool with either control oligonucleotides that do not cover known methylation sites (*e.g.*, a fluorescence-based version of the HeavyMethyl™ and MSP techniques) or with oligonucleotides covering potential methylation sites.

The MethyLight™ process is used with any suitable probe (*e.g.* a "TaqMan®" probe, a Lightcycler® probe, *etc.*) For example, in some applications double-stranded genomic DNA is treated with sodium bisulfite and subjected to one of two sets of PCR reactions using TaqMan® probes, *e.g.*, with MSP primers and/or HeavyMethyl blocker oligonucleotides and a TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules and is designed to be specific for a relatively high GC content region so that it melts at about a 10°C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the Taq polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5′ to 3′ endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system.

Typical reagents (*e.g.*, as might be found in a typical MethyLight™-based kit) for MethyLight™ analysis may include, but are not limited to: PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, *etc.*); TaqMan® or Lightcycler® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

The QM™ (quantitative methylation) assay is an alternative quantitative test for methylation patterns in genomic DNA samples, wherein sequence discrimination occurs at the level of probe hybridization. In this quantitative version, the PCR reaction provides for unbiased amplification in the presence of a fluorescent probe that overlaps a particular putative methylation site. An unbiased control for the amount of input DNA is provided by a reaction in which neither the primers, nor the probe, overlie any CpG dinucleotides. Alternatively, a qualitative test for genomic methylation is achieved by probing the biased PCR pool with either control oligonucleotides that do not cover known methylation sites (a fluorescence-based version of the HeavyMethyl™ and MSP techniques) or with oligonucleotides covering potential methylation sites.

The QM™ process can be used with any suitable probe, *e.g.*, "TaqMan®" probes, Lightcycler® probes, in the amplification process. For example, double-stranded genomic DNA is treated with sodium bisulfite and subjected to unbiased primers and the TaqMan® probe. The TaqMan® probe is dual-labeled with fluorescent "reporter" and "quencher" molecules, and is designed to be specific for a relatively high GC content region so that it melts out at about a 10°C higher temperature in the PCR cycle than the forward or reverse primers. This allows the TaqMan® probe to remain fully hybridized during the PCR annealing/extension step. As the Taq polymerase enzymatically synthesizes a new strand during PCR, it will eventually reach the annealed TaqMan® probe. The Taq polymerase 5′ to 3′ endonuclease activity will then displace the TaqMan® probe by digesting it to release the fluorescent reporter molecule for quantitative detection of its now unquenched signal using a real-time fluorescent detection system. Typical reagents (*e.g.*, as might be found in a typical QM™-based kit) for QM™ analysis may include, but are not limited to: PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, *etc.*); TaqMan® or Lightcycler® probes; optimized PCR buffers and deoxynucleotides; and Taq polymerase.

The Ms-SNuPE™ technique is a quantitative method for assessing methylation differences at specific CpG sites based on bisulfite treatment of DNA, followed by single-nucleotide primer extension (Gonzalgo & Jones, Nucleic Acids Res. 25:2529-2531, 1997). Briefly, genomic DNA is reacted with sodium bisulfite to convert unmethylated cytosine to uracil while leaving 5-methylcytosine unchanged. Amplification of the desired target sequence is then performed using PCR primers specific for bisulfite-converted DNA, and the resulting product is isolated and used as a template for methylation analysis at the CpG site of interest. Small amounts of DNA can be analyzed (*e.g.*, microdissected pathology sections) and it avoids utilization of restriction enzymes for determining the methylation status at CpG sites.

Typical reagents (*e.g.*, as might be found in a typical Ms-SNuPE™-based kit) for Ms-SNuPE™ analysis may include, but are not limited to: PCR primers for specific loci (*e.g.*, specific genes, markers, regions of genes, regions of markers, bisulfite treated DNA sequence, CpG island, *etc.*); optimized PCR buffers and deoxynucleotides; gel extraction kit; positive control primers; Ms-SNuPE™ primers for specific loci; reaction buffer (for the Ms-SNuPE reaction); and labeled nucleotides. Additionally, bisulfite conversion reagents may include: DNA denaturation buffer; sulfonation buffer; DNA recovery reagents or kit (*e.g.*,

precipitation, ultrafiltration, affinity column); desulfonation buffer; and DNA recovery components.

Reduced Representation Bisulfite Sequencing (RRBS) begins with bisulfite treatment of nucleic acid to convert all unmethylated cytosines to uracil, followed by restriction enzyme digestion (*e.g.*, by an enzyme that recognizes a site including a CG sequence such as MspI) and complete sequencing of fragments after coupling to an adapter ligand. The choice of restriction enzyme enriches the fragments for CpG dense regions, reducing the number of redundant sequences that may map to multiple gene positions during analysis. As such, RRBS reduces the complexity of the nucleic acid sample by selecting a subset (*e.g.*, by size selection using preparative gel electrophoresis) of restriction fragments for sequencing. As opposed to whole-genome bisulfite sequencing, every fragment produced by the restriction enzyme digestion contains DNA methylation information for at least one CpG dinucleotide. As such, RRBS enriches the sample for promoters, CpG islands, and other genomic features with a high frequency of restriction enzyme cut sites in these regions and thus provides an assay to assess the methylation state of one or more genomic loci.

A typical protocol for RRBS comprises the steps of digesting a nucleic acid sample with a restriction enzyme such as MspI, filling in overhangs and A-tailing, ligating adaptors, bisulfite conversion, and PCR. See, *e.g.*, et al. (2005) "Genome-scale DNA methylation mapping of clinical samples at single-nucleotide resolution" *Nat Methods* **7**: 133–6; Meissner et al. (2005) "Reduced representation bisulfite sequencing for comparative high-resolution DNA methylation analysis" *Nucleic Acids Res.* **33**: 5868–77.

In some embodiments, a quantitative allele-specific real-time target and signal amplification (QuARTS) assay is used to evaluate methylation state. Three reactions sequentially occur in each QuARTS assay, including amplification (reaction 1) and target probe cleavage (reaction 2) in the primary reaction; and FRET cleavage and fluorescent signal generation (reaction 3) in the secondary reaction. When target nucleic acid is amplified with specific primers, a specific detection probe with a flap sequence loosely binds to the amplicon. The presence of the specific invasive oligonucleotide at the target binding site causes a 5′ nuclease, *e.g.*, a FEN-1 endonuclease, to release the flap sequence by cutting between the detection probe and the flap sequence. The flap sequence is complementary to a non-hairpin portion of a corresponding FRET cassette. Accordingly, the flap sequence functions as an invasive oligonucleotide on the FRET cassette and effects a cleavage between the FRET cassette fluorophore and a quencher, which produces a fluorescent signal. The

cleavage reaction can cut multiple probes per target and thus release multiple fluorophore per flap, providing exponential signal amplification. QuARTS can detect multiple targets in a single reaction well by using FRET cassettes with different dyes. See, *e.g.*, in Zou et al. (2010) "Sensitive quantification of methylated markers with a novel methylation specific technology" *Clin Chem* **56**: A199), and U.S. Pat. Nos. 8,361,720; 8,715,937; 8,916,344; and 9,212,392, each of which is incorporated herein by reference for all purposes.

In some embodiments, the bisulfite-treated DNA is purified prior to the quantification. This may be conducted by any means known in the art, such as but not limited to ultrafiltration, *e.g.*, by means of Microcon™ columns (manufactured by Millipore™). The purification is carried out according to a modified manufacturer's protocol (see, *e.g.*, PCT/EP2004/011715, which is incorporated by reference in its entirety). In some embodiments, the bisulfite treated DNA is bound to a solid support, *e.g.*, a magnetic bead, and desulfonation and washing occurs while the DNA is bound to the support. Examples of such embodiments are provided, *e.g.*, in WO 2013/116375 and U.S. Pat. No. 9,315,853. In certain preferred embodiments, support-bound DNA is ready for a methylation assay immediately after desulfonation and washing on the support. In some embodiments, the desulfonated DNA is eluted from the support prior to assay.

In some embodiments, fragments of the treated DNA are amplified using sets of primer oligonucleotides according to the present invention (*e.g.*, see Figure 1) and an amplification enzyme. The amplification of several DNA segments can be carried out simultaneously in one and the same reaction vessel. Typically, the amplification is carried out using a polymerase chain reaction (PCR).

Methods for isolating DNA suitable for these assay technologies are known in the art. In particular, some embodiments comprise isolation of nucleic acids as described in U.S. Pat. Nos. 9,000,146 and 9,163,278, each incorporated herein by reference in its entirety.

In some embodiments, the markers described herein find use in QUARTS assays performed on stool samples. In some embodiments, methods for producing DNA samples and, in particular, to methods for producing DNA samples that comprise highly purified, low-abundance nucleic acids in a small volume (*e.g.*, less than 100, less than 60 microliters) and that are substantially and/or effectively free of substances that inhibit assays used to test the DNA samples (*e.g.*, PCR, INVADER, QuARTS assays, *etc.*) are provided. Such DNA samples find use in diagnostic assays that qualitatively detect the presence of, or

quantitatively measure the activity, expression, or amount of, a gene, a gene variant (*e.g.*, an allele), or a gene modification (*e.g.*, methylation) present in a sample taken from a patient. For example, some cancers are correlated with the presence of particular mutant alleles or particular methylation states, and thus detecting and/or quantifying such mutant alleles or methylation states has predictive value in the diagnosis and treatment of cancer.

Many valuable genetic markers are present in extremely low amounts in samples and many of the events that produce such markers are rare. Consequently, even sensitive detection methods such as PCR require a large amount of DNA to provide enough of a low-abundance target to meet or supersede the detection threshold of the assay. Moreover, the presence of even low amounts of inhibitory substances compromise the accuracy and precision of these assays directed to detecting such low amounts of a target. Accordingly, provided herein are methods providing the requisite management of volume and concentration to produce such DNA samples.

In some embodiments, the sample comprises blood, serum, plasma, or saliva. In some embodiments, the subject is human. Such samples can be obtained by any number of means known in the art, such as will be apparent to the skilled person. Cell free or substantially cell free samples can be obtained by subjecting the sample to various techniques known to those of skill in the art which include, but are not limited to, centrifugation and filtration. Although it is generally preferred that no invasive techniques are used to obtain the sample, it still may be preferable to obtain samples such as tissue homogenates, tissue sections, and biopsy specimens. The technology is not limited in the methods used to prepare the samples and provide a nucleic acid for testing. For example, in some embodiments, a DNA is isolated from a stool sample or from blood or from a plasma sample using direct gene capture, *e.g.*, as detailed in U.S. Pat. Nos. 8,808,990 and 9,169,511, and in WO 2012/155072, or by a related method.

The analysis of markers can be carried out separately or simultaneously with additional markers within one test sample. For example, several markers can be combined into one test for efficient processing of multiple samples and for potentially providing greater diagnostic and/or prognostic accuracy. In addition, one skilled in the art would recognize the value of testing multiple samples (for example, at successive time points) from the same subject. Such testing of serial samples can allow the identification of changes in marker methylation states over time. Changes in methylation state, as well as the absence of change in methylation state, can provide useful information about the disease status that includes, but

is not limited to, identifying the approximate time from onset of the event, the presence and amount of salvageable tissue, the appropriateness of drug therapies, the effectiveness of various therapies, and identification of the subject's outcome, including risk of future events.

The analysis of biomarkers can be carried out in a variety of physical formats. For example, the use of microtiter plates or automation can be used to facilitate the processing of large numbers of test samples. Alternatively, single sample formats could be developed to facilitate immediate treatment and diagnosis in a timely fashion, for example, in ambulatory transport or emergency room settings.

It is contemplated that embodiments of the technology are provided in the form of a kit. The kits comprise embodiments of the compositions, devices, apparatuses, *etc.* described herein, and instructions for use of the kit. Such instructions describe appropriate methods for preparing an analyte from a sample, *e.g.*, for collecting a sample and preparing a nucleic acid from the sample. Individual components of the kit are packaged in appropriate containers and packaging (*e.g.*, vials, boxes, blister packs, ampules, jars, bottles, tubes, and the like) and the components are packaged together in an appropriate container (*e.g.*, a box or boxes) for convenient storage, shipping, and/or use by the user of the kit. It is understood that liquid components (*e.g.*, a buffer) may be provided in a lyophilized form to be reconstituted by the user. Kits may include a control or reference for assessing, validating, and/or assuring the performance of the kit. For example, a kit for assaying the amount of a nucleic acid present in a sample may include a control comprising a known concentration of the same or another nucleic acid for comparison and, in some embodiments, a detection reagent (*e.g.*, a primer) specific for the control nucleic acid. The kits are appropriate for use in a clinical setting and, in some embodiments, for use in a user's home. The components of a kit, in some embodiments, provide the functionalities of a system for preparing a nucleic acid solution from a sample. In some embodiments, certain components of the system are provided by the user.

III. Applications

In some embodiments, diagnostic assays identify the presence of a disease or condition in an individual. In some embodiments, the disease is cancer (*e.g.*, lung cancer). In some embodiments, markers whose aberrant methylation is associated with a lung cancer (*e.g.*, one or more markers selected from the markers listed in Table 1, or preferably one or

more of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, , BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF329, IFFO1,* and *HOPX*) are used. In some embodiments, an assay further comprises detection of a reference gene (*e.g.*, β-actin, *ZDHHC1, B3GALT6.* See, *e.g.*, U.S. Patent Appln. No. 14/966,617, filed 12/11/2015, and U.S. Pat. Appl. No. 62/364,082, filed 07/19/2016, each of which is incorporated herein by reference for all purposes).

In some embodiments, the technology finds application in treating a patient (*e.g.*, a patient with lung cancer, with early stage lung cancer, or who may develop lung cancer), the method comprising determining the methylation state of one or more markers as provided herein and administering a treatment to the patient based on the results of determining the methylation state. The treatment may be administration of a pharmaceutical compound, a vaccine, performing a surgery, imaging the patient, performing another test. Preferably, said use is in a method of clinical screening, a method of prognosis assessment, a method of monitoring the results of therapy, a method to identify patients most likely to respond to a particular therapeutic treatment, a method of imaging a patient or subject, and a method for drug screening and development.

In some embodiments, the technology finds application in methods for diagnosing lung cancer in a subject is provided. The terms “diagnosing” and “diagnosis” as used herein refer to methods by which the skilled artisan can estimate and even determine whether or not a subject is suffering from a given disease or condition or may develop a given disease or condition in the future. The skilled artisan often makes a diagnosis on the basis of one or more diagnostic indicators, such as for example a biomarker, the methylation state of which is indicative of the presence, severity, or absence of the condition.

Along with diagnosis, clinical cancer prognosis relates to determining the aggressiveness of the cancer and the likelihood of tumor recurrence to plan the most effective therapy. If a more accurate prognosis can be made or even a potential risk for developing the cancer can be assessed, appropriate therapy, and in some instances less severe therapy for the

patient can be chosen. Assessment (*e.g.*, determining methylation state) of cancer biomarkers is useful to separate subjects with good prognosis and/or low risk of developing cancer who will need no therapy or limited therapy from those more likely to develop cancer or suffer a recurrence of cancer who might benefit from more intensive treatments.

As such, "making a diagnosis" or "diagnosing", as used herein, is further inclusive of making determining a risk of developing cancer or determining a prognosis, which can provide for predicting a clinical outcome (with or without medical treatment), selecting an appropriate treatment (or whether treatment would be effective), or monitoring a current treatment and potentially changing the treatment, based on the measure of the diagnostic biomarkers disclosed herein.

Further, in some embodiments of the technology, multiple determinations of the biomarkers over time can be made to facilitate diagnosis and/or prognosis. A temporal change in the biomarker can be used to predict a clinical outcome, monitor the progression of lung cancer, and/or monitor the efficacy of appropriate therapies directed against the cancer. In such an embodiment for example, one might expect to see a change in the methylation state of one or more biomarkers disclosed herein (and potentially one or more additional biomarker(s), if monitored) in a biological sample over time during the course of an effective therapy.

The technology further finds application in methods for determining whether to initiate or continue prophylaxis or treatment of a cancer in a subject. In some embodiments, the method comprises providing a series of biological samples over a time period from the subject; analyzing the series of biological samples to determine a methylation state of at least one biomarker disclosed herein in each of the biological samples; and comparing any measurable change in the methylation states of one or more of the biomarkers in each of the biological samples. Any changes in the methylation states of biomarkers over the time period can be used to predict risk of developing cancer, predict clinical outcome, determine whether to initiate or continue the prophylaxis or therapy of the cancer, and whether a current therapy is effectively treating the cancer. For example, a first time point can be selected prior to initiation of a treatment and a second time point can be selected at some time after initiation of the treatment. Methylation states can be measured in each of the samples taken from different time points and qualitative and/or quantitative differences noted. A change in the methylation states of the biomarker levels from the different samples can be correlated with

risk for developing lung, prognosis, determining treatment efficacy, and/or progression of the cancer in the subject.

In preferred embodiments, the methods and compositions of the invention are for treatment or diagnosis of disease at an early stage, for example, before symptoms of the disease appear. In some embodiments, the methods and compositions of the invention are for treatment or diagnosis of disease at a clinical stage.

As noted above, in some embodiments multiple determinations of one or more diagnostic or prognostic biomarkers can be made, and a temporal change in the marker can be used to determine a diagnosis or prognosis. For example, a diagnostic marker can be determined at an initial time, and again at a second time. In such embodiments, an increase in the marker from the initial time to the second time can be diagnostic of a particular type or severity of cancer, or a given prognosis. Likewise, a decrease in the marker from the initial time to the second time can be indicative of a particular type or severity of cancer, or a given prognosis. Furthermore, the degree of change of one or more markers can be related to the severity of the cancer and future adverse events. The skilled artisan will understand that, while in certain embodiments comparative measurements can be made of the same biomarker at multiple time points, one can also measure a given biomarker at one time point, and a second biomarker at a second time point, and a comparison of these markers can provide diagnostic information.

As used herein, the phrase "determining the prognosis" refers to methods by which the skilled artisan can predict the course or outcome of a condition in a subject. The term "prognosis" does not refer to the ability to predict the course or outcome of a condition with 100% accuracy, or even that a given course or outcome is predictably more or less likely to occur based on the methylation state of a biomarker. Instead, the skilled artisan will understand that the term "prognosis" refers to an increased probability that a certain course or outcome will occur; that is, that a course or outcome is more likely to occur in a subject exhibiting a given condition, when compared to those individuals not exhibiting the condition. For example, in individuals not exhibiting the condition, the chance of a given outcome (*e.g.*, suffering from lung cancer) may be very low.

In some embodiments, a statistical analysis associates a prognostic indicator with a predisposition to an adverse outcome. For example, in some embodiments, a methylation state different from that in a normal control sample obtained from a patient who does not

have a cancer can signal that a subject is more likely to suffer from a cancer than subjects with a level that is more similar to the methylation state in the control sample, as determined by a level of statistical significance. Additionally, a change in methylation state from a baseline (*e.g.*, "normal") level can be reflective of subject prognosis, and the degree of change in methylation state can be related to the severity of adverse events. Statistical significance is often determined by comparing two or more populations and determining a confidence interval and/or a *p* value. See, *e.g.*, Dowdy and Wearden, Statistics for Research, John Wiley & Sons, New York, 1983, incorporated herein by reference in its entirety. Exemplary confidence intervals of the present subject matter are 90%, 95%, 97.5%, 98%, 99%, 99.5%, 99.9% and 99.99%, while exemplary *p* values are 0.1, 0.05, 0.025, 0.02, 0.01, 0.005, 0.001, and 0.0001.

In other embodiments, a threshold degree of change in the methylation state of a prognostic or diagnostic biomarker disclosed herein can be established, and the degree of change in the methylation state of the biomarker in a biological sample is simply compared to the threshold degree of change in the methylation state. A preferred threshold change in the methylation state for biomarkers provided herein is about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 50%, about 75%, about 100%, and about 150%. In yet other embodiments, a "nomogram" can be established, by which a methylation state of a prognostic or diagnostic indicator (biomarker or combination of biomarkers) is directly related to an associated disposition towards a given outcome. The skilled artisan is acquainted with the use of such nomograms to relate two numeric values with the understanding that the uncertainty in this measurement is the same as the uncertainty in the marker concentration because individual sample measurements are referenced, not population averages.

In some embodiments, a control sample is analyzed concurrently with the biological sample, such that the results obtained from the biological sample can be compared to the results obtained from the control sample. Additionally, it is contemplated that standard curves can be provided, with which assay results for the biological sample may be compared. Such standard curves present methylation states of a biomarker as a function of assay units, *e.g.*, fluorescent signal intensity, if a fluorescent label is used. Using samples taken from multiple donors, standard curves can be provided for control methylation states of the one or more biomarkers in normal tissue, as well as for "at-risk" levels of the one or more biomarkers in tissue taken from donors with lung cancer.

The analysis of markers can be carried out separately or simultaneously with additional markers within one test sample. For example, several markers can be combined into one test for efficient processing of a multiple of samples and for potentially providing greater diagnostic and/or prognostic accuracy. In addition, one skilled in the art would recognize the value of testing multiple samples (for example, at successive time points) from the same subject. Such testing of serial samples can allow the identification of changes in marker methylation states over time. Changes in methylation state, as well as the absence of change in methylation state, can provide useful information about the disease status that includes, but is not limited to, identifying the approximate time from onset of the event, the presence and amount of salvageable tissue, the appropriateness of drug therapies, the effectiveness of various therapies, and identification of the subject's outcome, including risk of future events.

The analysis of biomarkers can be carried out in a variety of physical formats. For example, the use of microtiter plates or automation can be used to facilitate the processing of large numbers of test samples. Alternatively, single sample formats could be developed to facilitate immediate treatment and diagnosis in a timely fashion, for example, in ambulatory transport or emergency room settings.

In some embodiments, the subject is diagnosed as having lung cancer if, when compared to a control methylation state, there is a measurable difference in the methylation state of at least one biomarker in the sample. Conversely, when no change in methylation state is identified in the biological sample, the subject can be identified as not having lung cancer, not being at risk for the cancer, or as having a low risk of the cancer. In this regard, subjects having lung cancer or risk thereof can be differentiated from subjects having low to substantially no cancer or risk thereof. Those subjects having a risk of developing lung cancer can be placed on a more intensive and/or regular screening schedule. On the other hand, those subjects having low to substantially no risk may avoid being subjected to screening procedures, until such time as a future screening, for example, a screening conducted in accordance with the present technology, indicates that a risk of lung cancer has appeared in those subjects.

As mentioned above, depending on the embodiment of the method of the present technology, detecting a change in methylation state of the one or more biomarkers can be a qualitative determination or it can be a quantitative determination. As such, the step of diagnosing a subject as having, or at risk of developing, lung cancer indicates that certain

threshold measurements are made, *e.g.*, the methylation state of the one or more biomarkers in the biological sample varies from a predetermined control methylation state. In some embodiments of the method, the control methylation state is any detectable methylation state of the biomarker. In other embodiments of the method where a control sample is tested concurrently with the biological sample, the predetermined methylation state is the methylation state in the control sample. In other embodiments of the method, the predetermined methylation state is based upon and/or identified by a standard curve. In other embodiments of the method, the predetermined methylation state is a specifically state or range of state. As such, the predetermined methylation state can be chosen, within acceptable limits that will be apparent to those skilled in the art, based in part on the embodiment of the method being practiced and the desired specificity, etc.

In some embodiments, a sample from a subject having or suspected of having lung cancer is screened using one or more methylation markers and suitable assay methods that provide data that differentiate between different types of lung cancer, *e.g.*, non-small cell (adenocarcinoma, large cell carcinoma, squamous cell carcinoma) and small cell carcinomas. See, *e.g.*, marker ref. # AC27 (Fig 2; PLEC), which is highly methylated (shown as mean methylation compared to mean methylation at that locus in normal buffy coat) in adenocarcinoma and small cell carcinomas, but not in large cell or squamous cell carcinoma; marker ref. # AC23 (Fig 2; ITPRIPL1), which is more highly methylated in adenocarcinoma than in any other sample type; marker ref. # LC2 (Fig. 3; DOCK2)), which is more highly methylated in large cell carcinomas than in any other sample type; marker ref # SC221 (Fig. 4; ST8SIA4), which is more highly methylated in small cell carcinomas than in any other sample type; and marker ref. # SQ36 (Fig. 5, DOK1), which is more highly methylated in squamous cell carcinoma than in than in any other sample type.

Methylation markers selected as described herein may be used alone or in combination (*e.g.*, in panels) such that analysis of a sample from a subject reveals the presence of a lung neoplasm and also provides sufficient information to distinguish between lung cancer type, *e.g.*, small cell carcinoma vs. non-small cell carcinoma. In preferred embodiments, a marker or combination of markers further provide data sufficient to distinguish between adenomcarcinomas, large cell carcinomas, and squamous cell carcinomas; and/or to characterize carcinomas of undetermined or mixed pathologies. In other embodiments, methylation markers or combinations thereof are selected to provide a

positive result (*i.e.,* a result indicating the presence of lung neoplasm) regardless of the type of lung carcinoma present, without differentiating data.

Over recent years, it has become apparent that circulating epithelial cells, representing metastatic tumor cells, can be detected in the blood of many patients with cancer. Molecular profiling of rare cells is important in biological and clinical studies. Applications range from characterization of circulating epithelial cells (CEpCs) in the peripheral blood of cancer patients for disease prognosis and personalized treatment (See *e.g.*, Cristofanilli M, et al. (2004) N Engl J Med 351:781–791; Hayes DF, et al. (2006) Clin Cancer Res 12:4218–4224; Budd GT, et al., (2006) Clin Cancer Res 12:6403–6409; Moreno JG, et al. (2005) Urology 65:713–718; Pantel et al., (2008) Nat Rev 8:329–340; and Cohen SJ, et al. (2008) J Clin Oncol 26:3213–3221). Accordingly, embodiments of the present disclosure provide compositions and methods for detecting the presence of metastatic cancer in a subject by identifying the presence of methylation markers in plasma or whole blood.

EXPERIMENTAL EXAMPLES

EXAMPLE 1

Sample preparation methods

Methods for DNA Isolation and QUARTS Assay

The following provides exemplary method for DNA isolation prior to analysis, and an exemplary QUARTS assay, such as may be used in accordance with embodiments of the technology. Application of QuARTS technology to DNA from blood and various tissue samples is described in this example, but the technology is readily applied to other nucleic acid samples, as shown in other examples.

DNA isolation from cells and plasma

For cell lines, genomic DNA may be isolated from cell conditioned media using, for example, the "Maxwell® RSC ccfDNA Plasma Kit (Promega Corp., Madison, WI). Following the kit protocol, 1 mL of cell conditioned media (CCM) is used in place of plasma, and processed according to the kit procedure. The elution volume is 100 µL, of which 70 µL are generally used for bisulfite conversion.

An exemplary procedure for isolating DNA from a 4 mL sample of plasma is as follows:

- To a 4 mL sample of plasma, 300 µL of Proteinase K (20mg/mL) is added and mixed.
- Add 3 µL of 1 µg/µL of Fish DNA to the plasma-proteinase K mixture.
- Add 2 mL of plasma lysis buffer to plasma.

  Plasma lysis buffer is:

  - 4.3M guanidine thiocyanate
  - 10% IGEPAL CA-630 (Octylphenoxy poly(ethyleneoxy)ethanol, branched)

  (5.3g of IGEPAL CA-630 combined with 45 mL of 4.8 M guanidine thiocyanate)
- Incubate mixtures at 55°C for 1 hour with shaking at 500 rpm.
- Add and mix:
  - 3 mL of plasma lysis buffer
  - 200 µL magnetic silica binding beads (16 µg of beads/µL)
  - Add 2 mL of 100% isopropanol

  (optionally mix after each addition and/or optionally pre-mix the lysis buffer and isopropanol before adding to the mixture)
- Incubate at 30°C for 30 minutes with shaking at 500 rpm.
- Place tube(s) on magnet and let the beads collect. Aspirate and discard the supernatant.
- Add 750µL GuHCl-EtOH to vessel containing the binding beads and mix.

  GuHCl-EtOH wash buffer is:

  - 3M GuHCl (guanidine hydrochloride)
  - 57% EtOH (ethyl alcohol)
- Shake at 400 rpm for 1 minute.
- Transfer samples to a deep well plate or 2 mL microcentrifuge tubes.
- Place tubes on magnet and let the beads collect for 10 minutes. Aspirate and discard the supernatant.
- Add 1000 µL wash buffer (10 mM Tris HCl, 80% EtOH) to the beads, and incubate at 30°C for 3 minutes with shaking.

- Place tubes on magnet and let the beads collect. Aspirate and discard the supernatant.
- Add 500 µL wash buffer to the beads and incubate at 30°C for 3 minutes with shaking.
- Place tubes on magnet and let the beads collect. Aspirate and discard the supernatant.
- Add 250 µL wash buffer and incubate at 30°C for 3 minutes with shaking.
- Place tubes on magnet and let the beads collect. Aspirate and discard the remaining buffer.
- Add 250 µL wash buffer and incubate at 30°C for 3 minutes with shaking.
- Place tubes on magnet and let the beads collect. Aspirate and discard the remaining buffer.
- Dry the beads at 70°C for 15 minutes, with shaking.
- Add 125 µL elution buffer (10 mM Tris HCl, pH 8.0, 0.1 mM EDTA) to the beads and incubate at 65°C for 25 minutes with shaking.
- Place tubes on magnet and let the beads collect for 10 minutes.
- Aspirate and transfer the supernatant containing the DNA to a new vessel or tube.

Bisulfite conversion

I. Sulfonation of DNA using ammonium hydrogen sulfite

1. In each tube, combine 64 µL DNA, 7 µL 1 N NaOH, and 9 µL of carrier solution containing 0.2 mg/mL BSA and 0.25 mg/mL of fish DNA.
2. Incubate at 42°C for 20 minutes.
3. Add 120 µL of 45% ammonium hydrogen sulfite and incubate at 66° for 75 minutes.
4. Incubate at 4°C for 10 minutes.

II. Desulfonation using magnetic beads

Materials

- Magnetic beads (Promega MagneSil Paramagnetic Particles, Promega catalogue number AS1050, 16 µg/µL).
- Binding buffer: 6.5-7 M guanidine hydrochoride.
- Post-conversion Wash buffer: 80% ethanol with 10 mM Tris HCl (pH 8.0).

- Desulfonation buffer: 70% isopropyl alcohol, 0.1 N NaOH was selected for the desulfonation buffer.

Samples are mixed using any appropriate device or technology to mix or incubate samples at the temperatures and mixing speeds essentially as described below. For example, a Thermomixer (Eppendorf) can be used for the mixing or incubation of samples. An exemplary desulfonation is as follows:

1. Mix bead stock thoroughly by vortexing bottle for 1 minute.
2. Aliquot 50 µL of beads into a 2.0 mL tube (*e.g.*, from USA Scientific).
3. Add 750 µL of binding buffer to the beads.
4. Add 150 µL of sulfonated DNA from step I.
5. Mix (*e.g.*, 1000 RPM at 30°C for 30 minutes).
6. Place tube on the magnet stand and leave in place for 5 minutes. With the tubes on the stand, remove and discard the supernatant.
7. Add 1,000 µL of wash buffer. Mix (*e.g.*, 1000 RPM at 30°C for 3 minutes).
8. Place tube on the magnet stand and leave in place for 5 minutes. With the tubes on the stand, remove and discard the supernatant.
9. Add 250 µL of wash buffer. Mix (*e.g.*, 1000 RPM at 30°C for 3 minutes).
10. Place tube on magnetic rack; remove and discard supernatant after 1 minute.
11. Add 200 µL of desulfonation buffer. Mix (*e.g.*, 1000 RPM at 30°C for 5 minutes).
12. Place tube on magnetic rack; remove and discard supernatant after 1 minute.
13. Add 250 µL of wash buffer. Mix (*e.g.*, 1000 RPM at 30°C for 3 minutes).
14. Place tube on magnetic rack; remove and discard supernatant after 1 minute.
15. Add 250 µL of wash buffer to the tube. Mix (*e.g.*, 1000 RPM at 30°C for 3 minutes).
16. Place tube on magnetic rack; remove and discard supernatant after 1 minute.
17. Incubate all tubes at 30°C with the lid open for 15 minutes.
18. Remove tube from magnetic rack and add 70 µL of elution buffer directly to the beads.
19. Incubate the beads with elution-buffer (*e.g.*, 1000 RPM at 40°C for 45 minutes).
20. Place tubes on magnetic rack for about one minute; remove and save the supernatant.

The converted DNA is then used in a detection assay, *e.g.*, a pre-amplification and/or flap endonuclease assays, as described below.

See also U.S. Patent Appl. Ser. Nos. 62/249,097, filed October 30, 2015; 15/335,111 and 15/335,096, both filed October 26, 2016; and International Appl. Ser. No. PCT/US16/58875, filed October 26, 2016, each of which is incorporated herein by reference in its entirety, for all purposes.

QuARTS assay

The QuARTS technology combines a polymerase-based target DNA amplification process with an invasive cleavage-based signal amplification process. The technology is described, *e.g.*, in U.S. Pat. Nos. 8,361,720; 8,715,937; 8,916,344; and 9,212,392, and U.S. Pat. Appl. No. 15/841,006, each of which is incorporated herein by reference. Fluorescence signal generated by the QuARTS reaction is monitored in a fashion similar to real-time PCR and permits quantitation of the amount of a target nucleic acid in a sample.

An exemplary QuARTS reaction typically comprises approximately 400–600 nmol/L (*e.g.*, 500 nmol/L) of each primer and detection probe, approximately 100 nmol/L of the invasive oligonucleotide, approximately 600–700 nmol/L of each FRET cassette (FAM, *e.g.*, as supplied commercially by Hologic, Inc.; HEX, *e.g.*, as supplied commercially by BioSearch Technologies; and Quasar 670, *e.g.*, as supplied commercially by BioSearch Technologies), 6.675 ng/µL FEN-1 endonuclease (*e.g.*, Cleavase® 2.0, Hologic, Inc.), 1 unit Taq DNA polymerase in a 30 µL reaction volume (*e.g.*, GoTaq® DNA polymerase, Promega Corp., Madison ,WI), 10 mmol/L 3-(n-morpholino) propanesulfonic acid (MOPS), 7.5 mmol/L $MgCl_2$, and 250 µmol/L of each dNTP. Exemplary QuARTS cycling conditions are as shown in the table below. In some applications, analysis of the quantification cycle (C_q) provides a measure of the initial number of target DNA strands (*e.g.*, copy number) in the sample.

Stage	Temp/Time	# of Cycles
Denaturation	95°C /3'	1
Amplification 1	95°C / 20"	10
	67°C / 30"	
	70°C / 30"	
Amplification 2	95°C / 20"	37
	53°C / 1'	
	70°C / 30"	
Cooling	40°C / 30"	1

Multiplex Targeted Pre-amplification of Large-Volume Bisulfite-Converted DNA

To pre-amplify most or all of the bisulfite-treated DNA from an input sample, a large volume of the treated DNA may be used in a single, large-volume multiplex amplification reaction. For example, DNA is extracted from a cell lines (*e.g.*, DFCI032 cell line (adenocarcinoma); H1755 cell line (neuroendocrine), using, for example, the Maxwell Promega blood kit # AS1400, as described above. The DNA is bisulfite converted, *e.g.*, as described above.

A pre-amplification is conducted, for example, in a reaction mixture containing 7.5 mM $MgCl_2$, 10 mM MOPS, 0.3 mM Tris-HCl, pH 8.0, 0.8 mM KCl, 0.1 µg/µL BSA, 0.0001% Tween-20, 0. 0001% IGEPAL CA-630, 250 µM each dNTP, oligonucleotide primers, (*e.g.*, for 12 targets, 12 primer pairs/24 primers, in equimolar amounts (including but not limited to the ranges of, *e.g.,* 200-500 nM each primer), or with individual primer concentrations adjusted to balance amplification efficiencies of the different target regions), 0.025 units/µL HotStart GoTaq concentration, and 20 to 50% by volume of bisulfite-treated target DNA (*e.g.*, 10 µL of target DNA into a 50 µL reaction mixture, or 50 µL of target DNA into a 125 µL reaction mixture). Thermal cycling times and temperatures are selected to be appropriate for the volume of the reaction and the amplification vessel. For example, the reactions may be cycled as follows

Stage	Temp / Time	#of Cycles
Pre-incubation	95°C /5'	1
Amplification 1	95°C / 30"	10-12
	64°C / 30"	
	72°C / 30"	
Cooling	4°C / Hold	1

After thermal cycling, aliquots of the pre-amplification reaction (*e.g.*, 10 µL) are diluted to 500 µL in 10 mM Tris, 0.1 mM EDTA, with or without fish DNA. Aliquots of the diluted pre-amplified DNA (*e.g.*, 10 µL) are used in a QuARTS PCR-flap assay, *e.g.*, as described above. See also U.S. Patent Appl. Ser. No. 62/249,097, filed October 30, 2015; Appl. Ser No. 15/335,096, filed October 26, 2016, and PCT/US16/58875, filed October 26, 2016, each of which is incorporated herein by reference in its entirety for all purposes.

EXAMPLE 2

Selection and Testing of Methylation Markers

Marker selection process:

Reduced Representation Bisulfite Sequencing (RRBS) data was obtained on tissues from 16 adenocarcinoma lung cancer, 11 large cell lung cancer, 14 small cell lung cancer, 24 squamous cell lung cancer, and 18 non-cancer lung as well as RRBS results of buffy coat samples obtained from 26 healthy patients.

After alignment to a bisulfite-converted form of the human genome sequence, average methylation at each CpG island was computed for each sample type (i.e., tissue or buffy coat) and marker regions were selected based on the following criteria:

- Regions were selected to be 50 base pairs or longer.
- For QuARTS flap assay designs, regions were selected to have a minimum of 1 methylated CpG under each of: a) the probe region, b) the forward primer binding region, and c) the reverse primer binding region. For the forward and reverse primers, it is preferred that the methylated CpGs are close to the 3′-ends of the primers, but not at the 3’terminal nucleotide. Exemplary flap endonuclease assay oligonucleotides are shown in Figure 1.
- Preferably, buffy coat methylation at any CpG in a region of interest is no more than > 0.5%.
- Preferably, cancer tissue methylation in a region of interest is > 10%.
- For assays designed for tissue analysis, normal tissue methylation in a region of interest is preferably <0.5%.

RRBS data for different lung cancer tissue types is shown in Figs. 2-5. Based on the criteria above, the markers shown in the table below were selected and QuARTS flap assays were designed for them, as shown in Figure 1.

TABLE 1

Marker Name	Genomic coordinates
AGRN	chr1:968467-968582, strand=+
ANGPT1	chr8:108509559-108509684, strand=-
ANKRD13B	chr17:27940470-27940578, strand=+
ARHGEF4	chr2:131792758-131792900, strand=-

B3GALT6	chr1: 1163595-1163733, strand=+
BARX1	chr9:96721498-96721597, strand=-
BCAT1	chr12:25055868-25055986, strand=-
BCL2L11	chr2: 111876620-111876759, strand=-
BHLHE23	chr20:61638462-61638546, strand=-
BIN2	chr12:51717898-51717971, strand=-
BIN2_Z	chr12:51718088-51718165, strand=+
CAPN2	chr1:223936858-223936998, strand=+
chr17_737	chr17:73749814-73749919, strand=-
chr5_132	chr5:132161371-132161482,Strand=+
chr7_636	chr7:104581684-104581817, Strand=-
CYP26C1	chr10: 94822396-94822502, strand=+
DIDO1	chr20:61560669-61560753, strand=-
DLX4	chr17:48042426-48042820, strand=-
DMRTA2	chr1:50884390-50884519, strand=-
DNMT3A	chr2:25499967-25500072, strand=-
DOCK2	chr5:169064370- 169064454, strand=-
EMX1	chr2: 73147685-73147792, strand=+
FAM59B	chr2:26407701-26407828, strand=+
FERMT3	chr11:63974820-63974959, strand=+
FGF14	chr13:103046888-103046991, strand=+
FLJ34208	chr3:194208249-194208355, strand=+
FLJ45983	chr10:8097592-8097699, strand=+
GRIN2D	chr19:48918160-48918300, strand=-
HIST1H2BE	chr6:26184248-26184340, strand=+
HOPX	chr4:57521932-57522261 5'pad=0 3'pad=0 strand=-
IFFO1	chr12:6665277-6665348 strand=+
HOXA9	chr7:27205002-27205102, strand=-
HOXB2	chr17:46620545-46620639, strand=-
KLHDC7B	chr22: 50987199-50987256, strand=+
LOC100129726	chr2:43451705-43451810, strand=+
MATK	chr19:3786127-3786197, strand=+
MAX.chr10.22541891-22541946	chr10:22541881-22541975, strand=+
MAX.chr10.22624430-22624544	chr10:22624411-22624553, strand=-
MAX.chr12.52652268-52652362	chr12:52652262-52652377, strand=-
MAX.chr16.50875223-50875241	chr16:50875167-50875274, strand=-
MAX.chr19.16394489-16394575	chr19:16394457-16394593, strand=-
MAX.chr19.37288426-37288480	range=chr19:37288396-37288512, strand=-
MAX.chr8.124173236-124173370	chr8:124173231-124173386, strand=-
MAX.chr8.145105646-145105653	chr8:145105572-145105685, strand=-
MAX_Chr1.110	chr1:110627118-110627224 strand=-
NFIX	chr19:13207426-13207513, strand=+
NKX2-6	chr8:23564052-23564145, strand=-

OPLAH	chr8:145106777-145106865, strand=-
PARP15	chr3:122296692-122296805, strand=+
PRDM14	chr8:70981945-70982039, strand=-
PRKAR1B	chr7:644172-644237, strand=+
PRKCB_28	chr16:23847607-23847698, strand=-
PTGDR	chr14:52735270-52735400, strand=-
PTGDR_9	chr14:52735221-52735300, strand=+
RASSF1	chr3:50378408-50378550, strand=-
SHOX2	chr3:157821263-157821382, strand=-
SHROOM1	chr5:132161371-132161425, strand=+
SIPR4	chr19:3179921-3180068 strand=-
SKI	chr1:2232328-2232423, strand=+
SLC12A8	chr3:124860704-124860791, strand=+
SOBP	chr6: 107956176-107956234, strand=+
SP9	chr2:175201210-175201341, strand=-
SPOCK2	chr10:73847236-73847324, strand=-
ST8SIA1	chr12:22487518-22487630, strand=+
ST8SIA1_22	chr12:22486873-22487009, strand=-
SUCLG2	chr3:67706477-677065610, strand=-
TBX15 Region 1	chr1:119527066-119527655, strand=+
TBX15 Region 2	chr1:119532813-119532920 strand=-
TRH	chr3:129693481-129693580, strand=+
TSC22D4	chr7:100075328-100075445, strand=-
ZDHHC1	chr16:67428559-67428628, strand=-
ZMIZ1	chr10:81002910-81003005, strand=+
ZNF132	chr19:58951403-58951529, strand=-
ZNF329	chr19: 58661889- 58662028, strand=-
ZNF671	chr19:58238790-58238906, strand=+
ZNF781	ch19 : 38183018-38183137, strand=-

Analyzing selected markers for cross-reactivity with buffy coat.

1) Buffy coat screening

Markers from the list above were screened on DNA extracted from buffy coat obtained from 10 mL blood of a healthy patient. DNA was extracted using Promega Maxwell RSC system (Promega Corp., Fitchburg, WI) and converted using Zymo EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA). Using biplexed reaction with bisulfite-converted β-actin DNA ("BTACT"), and using approximately 40,000 strands of target genomic DNA, the samples were tested using a QuARTS flap endonuclease assay as described above, to test for cross reactivity. Doing so, the assays for 3 markers showed significant cross reactivity:

Marker	% Cross reactivity
HIST1H2B	72.93%
chr7_636	3495.47%
chr5_132	0.20%

2) Tissue screening

264 tissue samples were obtained from various commercial and non-commercial sources (Asuragen, BioServe, ConversantBio, Cureline, Mayo Clinic, M D Anderson, and PrecisionMed), as shown below in Table 2.

No. of cases	Pathology	Subtype	Details
82	Normal	NA	68 smokers, 34 never smokers, 17 smoking unknown
37	Normal	benign nodule	
7	NSCLC	bronchioalveolar	
13	NSCLC	large cell	
2	NSCLC	neuroendocrine	
42	NSCLC	squamous cell	
68	NSCLC	adenocarcinomas	
4	SCLC	small cell	
9	NSCLC	carcinoid	

Tissue sections were examined by a pathologist, who circled histologically distinct lesions to direct the micro-dissection. Total nucleic acid extraction was performed using the Promega Maxwell RSC system. Formalin-fixed, paraffin-embedded (FFPE) slides were scraped and the DNA was extracted using the Maxwell® RSC DNA FFPE Kit (#AS1450) using the manufacturer's procedure but skipping the RNase treatment step. The same procedure was used for FFPE curls. For frozen punch biopsy samples, a modified procedure using the lysis buffer from the RSC DNA FFPE kit with the Maxwell® RSC Blood DNA kit (#AS1400) was utilized omitting the RNase step. Samples were eluted in 10 mM Tris, 0.1 mM EDTA, pH 8.5 and 10 uL were used to setup 6 multiplex PCR reactions.

The following multiplex PCR primer mixes were made at 10X concentration (10X=2 μM each primer):

- Multiplex PCR reaction 1 consisted of each of the following markers: BARX1, LOC100129726, SPOCK2, TSC22D4, PARP15, MAX.chr8.145105646-145105653,

ST8SIA1_22, ZDHHC1, BIN2_Z, SKI, DNMT3A, BCL2L11, RASSF1, FERMT3, and BTACT.

- Multiplex PCR reaction 2 consisted of each of the following markers: ZNF671, ST8SIA1, NKX6-2, SLC12A8, FAM59B, DIDO1, MAX_Chr1.110, AGRN, PRKCB_28, SOBP, and BTACT.
- Multiplex PCR reaction 3 consisted of each of the following markers: MAX.chr10.22624430-22624544, ZMIZ1, MAX.chr8.145105646-145105653, MAX.chr10.22541891-22541946, PRDM14, ANGPT1, MAX.chr16.50875223-50875241, PTGDR_9, ANKRD13B, DOCK2, and BTACT.
- Multiplex PCR reaction 4 consisted of each of the following markers: MAX.chr19.16394489-16394575, HOXB2, ZNF132, MAX.chr19.37288426-37288480, MAX.chr12.52652268-52652362, FLJ45983, HOXA9, TRH, SP9, DMRTA2, and BTACT.
- Multiplex PCR reaction 5 consisted of each of the following markers: EMX1, ARHGEF4, OPLAH, CYP26C1, ZNF781, DLX4, PTGDR, KLHDC7B, GRIN2D, chr17_737, and BTACT.
- Multiplex PCR reaction 6 consisted of each of the following markers: TBX15, MATK, SHOX2, BCAT1, SUCLG2, BIN2, PRKAR1B, SHROOM1, S1PR4, NFIX, and BTACT.

Each multiplex PCR reaction was setup to a final concentration of 0.2μM reaction buffer, 0.2μM each primer, 0.05μM Hotstart Go Taq (5U/μL), resulting in 40 μL of master mix that was combined with 10μL of DNA template for a final reaction volume of 50μL.

The thermal profile for the multiplex PCR entailed a pre-incubation stage of 95° for 5 minutes, 10 cycles of amplification at 95° for 30 seconds, 64° for 30 seconds, 72° for 30 seconds, and a cooling stage of 4° that was held until further processing. Once the multiplex PCR was complete, the PCR product was diluted 1:10 using a diluent of 20ng/μL of fish DNA (*e.g.*, in water or buffer, see US Pat. No. 9,212,392, incorporated herein by reference) and 10μL of diluted amplified sample were used for each QuARTS assay reaction.

Each QuARTS assay was configured in triplex form, consisting of 2 methylation markers and BTACT as the reference gene.

- From multiplex PCR product 1, the following 7 triplex QuARTS assays were run: (1) BARX1, LOC100129726, BTACT; (2) SPOCK2, TSC22D4, BTACT; (3) PARP15, MAXchr8145105646-145105653, BTACT; (4) ST8SIA1_22, ZDHHC1, BTACT; (5) BIN2_Z, SKI, BTACT; (6) DNMT3A, BCL2L11, BTACT; (7) RASSF1, FERMT3, and BTACT.
- From multiplex PCR product 2, the following 5 triplex QuARTS assays were run: (1) ZNF671, ST8SIA1, BTACT; (2) NKX6-2, SLC12A8, BTACT; (3) FAM59B, DIDO1, BTACT; (4) MAX_Chr1110, AGRN, BTACT; (5) PRKCB_28, SOBP, and BTACT.
- From multiplex PCR product 3, the following 5 triplex QuARTS assays were run: (1) MAXchr1022624430-22624544, ZMIZ1, BTACT; (2) MAXchr8145105646-145105653, MAXchr1022541891-22541946, BTACT; (3) PRDM14, ANGPT1, BTACT; (4) MAXchr1650875223-50875241, PTGDR_9, BTACT; (5) ANKRD13B, DOCK2, and BTACT.
- From multiplex PCR product 4, the following 5 triplex QuARTS assays were run: (1) MAXchr1916394489-16394575, HOXB2, BTACT; (2) ZNF132, MAXchr1937288426-37288480, BTACT; (3) MAXchr1252652268-52652362, FLJ45983, BTACT; (4) HOXA9, TRH, BTACT; (5) SP9, DMRTA2, and BTACT.
- From multiplex PCR product 5, the following 5 triplex QuARTS assays were run: (1) EMX1, ARHGEF4, BTACT; (2) OPLAH, CYP26C1, BTACT; (3) ZNF781, DLX4, BTACT; (4) PTGDR, KLHDC7B, BTACT; (5) GRIN2D, chr17_737, and BTACT.
- From multiplex PCR product 6, the following 5 triplex QuARTS assays were run: (1) TBX15, MATK, BTACT; (2) SHOX2, BCAT1, BTACT; (3) SUCLG2, BIN2, BTACT; (4) PRKAR1B, SHROOM1, BTACT; (5) S1PR4, NFIX, and BTACT.

3) Data Analysis:

For tissue data analysis, markers that were selected based on RRBS criteria with <0.5 % methylation in normal tissue and >10% methylation in cancer tissue were included. This resulted in 51 markers for further analysis.

To determine marker sensitivities, the following was performed:

1. % methylation for each marker was computed by dividing strand values obtained for that specific marker by the strand values of ACTB (β-actin).
2. The maximum %methylation for each marker was determined on normal tissue. This is defined as 100% specificity.
3. The cancer tissue positivity for each marker was determined as the number of cancer tissues that had greater than the maximum normal tissue % methylation for that marker.

The sensitivities for the 51 markers are shown below.

TABLE 2

		Cancer (N=136)		
Marker	Maximum % methylation for normal	# Negative	# Positive	sensitivity
BARX1	1.665	66	70	51%
LOC100129726	1.847	109	27	20%
SPOCK2	0.261	86	50	37%
TSC22D4	0.618	70	66	49%
MAX.chr8.124	0.293	45	91	67%
RASSF1	1.605	79	57	42%
ZNF671	0.441	73	63	46%
ST8SIA1	1.56	119	17	13%
NKX6_2	15.58	102	34	25%
FAM59B	0.433	85	51	38%
DIDO1	2.29	93	43	32%
MAX_Chr1.110	0.076	85	51	38%
AGRN	2.16	66	70	51%
SOBP	38.5	110	26	19%
MAX_chr10.226	0.7	52	84	62%
ZMIZ1	0.025	72	64	47%
MAX_chr8.145	5.56	57	79	58%
MAX_chr10.225	0.77	72	64	47%
PRDM14	0.22	35	101	74%
ANGPT1	1.6	99	37	27%
MAX.chr16.50	0.27	92	44	32%
PTGDR_9	4.62	82	54	40%
ANKRD13B	7.03	93	43	32%
DOCK2	0.001	71	65	48%
MAX_chr19.163	0.61	56	80	59%

ZNF132	1.3	83	53	39%
MAX chr19.372	0.676	79	57	42%
HOXA9	16.7	53	83	61%
TRH	2.64	61	75	55%
SP9	14.99	75	61	45%
DMRTA2	7.9	55	81	60%
ARHGEF4	7.41	113	23	17%
CYP26C1	39.2	101	35	26%
ZNF781	5.28	44	92	68%
PTGDR	6.13	76	60	44%
GRIN2D	16.1	113	23	17%
MATK	0.04	93	43	32%
BCAT1	0.64	75	61	45%
PRKCB_28	1.68	57	79	58%
ST8SIA_22	1.934	55	81	60%
FLJ45983	8.34	39	97	71%
DLX4	15.1	41	95	70%
SHOX2	7.48	32	104	76%
EMX1	11.34	34	102	75%
HOXB2	0.114	61	75	55%
MAX.chr12.526	5.58	34	102	75%
BCL2L11	10.7	44	92	68%
OPLAH	5.11	29	107	79%
PARP15	3.077	42	94	69%
KLHDC7B	8.86	38	98	72%
SLC12A8	0.883	34	102	75%

Combinations of markers may be used to increase specificity and sensitivity. For example, a combination of the 8 markers *SLC12A8, KLHDC7B, PARP15, OPLAH, BCL2L11, MAX.chr12.526, HOXB2,* and *EMX1* resulted in 98.5% sensitivity (134/136 cancers) for all of the cancer tissues tested, with 100% specificity.

In some embodiments, markers are selected for sensitive and specific detection associated with a particular type of lung cancer tissue, *e.g.*, adenocarcinoma, large cell carcinoma, squamous cell carcinoma, or small cell carcinoma, *e.g.*, by use of markers that show sensitivity and specificity for particular cancer types or combinations of types.

This panel of methylated DNA markers assayed on tissue achieves extremely high discrimination for all types of lung cancer while remaining negative in normal lung tissue and benign nodules. Assays for this panel of markers can be also be applied to blood or bodily fluid-based testing, and finds applications in, *e.g.*, lung cancer screening and discrimination of malignant from benign nodules.

EXAMPLE 3

Testing a 30-Marker Set on Plasma Samples

From the list of markers in Example 2, 30 markers were selected for use in testing DNA from plasma samples from 295 subjects (64 with lung cancer, 231 normal controls. DNA was extracted from 2 mL of plasma from each subject and treated with bisulfite as described in Example 1. Aliquots of the bisulfite-converted DNA were used in two multiplex QuARTS assays, as described in Example 1. The markers selected for analysis are:

1. BARX1
2. BCL2L11
3. BIN2_Z
4. CYP26C1
5. DLX4
6. DMRTA2
7. DNMT3A
8. EMX1
9. FERMT3
10. FLJ45983
11. HOXA9
12. KLHDC7B
13. MAX.chr10.22624430-22624544
14. MAX.chr12.52652268-52652362
15. MAX.chr8.124173236-124173370
16. MAX.chr8.145105646-145105653
17. NFIX
18. OPLAH
19. PARP15
20. PRKCB_28
21. S1PR4
22. SHOX2
23. SKI
24. SLC12A8
25. SOBP
26. SP9
27. SUCLG2
28. TBX15
29. ZDHHC1
30. ZNF781

The target sequences, bisulfite converted target sequences, and the assay oligonucleotides for these markers were as shown in Fig. 1. The primers and flap oligonucleotides (probes) used for each converted target were as follows:

TABLE 3

Marker	Oligonucleotide Name	Component	Sequence (5'-3')	SEQ ID NO:
BARX1	BARX1_FP	Forward Primer	CGTTAATTTGTTAGATAGAGGGCG	23
	BARX1_RP	Reverse Primer	ACGATCGTCCGAACAACC	24
	BARX1_PB_A5	Flap Oligo.	CCACGGACGCGCCTACGAAAA/3C6/	25
SLC12A8	SLC12A8_FP	Forward Primer	TTAGGAGGGTGGGGTTCG	289
	SLC12A8_RP	Reverse Primer	CTTTCCTCGCAAAACCGC	290
	SLC12A8_Pb_A1	Flap Oligo.	CCACGGACGGGAGGGCGTAGG/3C6/	291
PARP15	PARP15_FP	Forward Primer	GGTTGAGTTTGGGGTTCG	236
	PARP15_RP	Reverse Primer	CGTAACGTAAAATCTCTACGCCC	237
	PARP15_Pb_A5	Flap Oligo.	CCACGGACGCGCTCGAACTAC/3C6/	238
MAX.Chr8.124	MAX.Chr8.124_FP	Forward Primer	GGTTGAGGTTTTCGGGTTTTTAG	203
	MAX.Chr8.124_RP	Reverse Primer	CCTCCCCACGAAATCGC	204
	MAX.Chr8.124_Pb_A1	Flap Oligo.	CGCCGAGGGCGGGTTTTCGT/3C6/	205
SHOX2	SHOX2_FP	Forward Primer	GTTCGAGTTTAGGGGTAGCG	269
	SHOX2_RP	Reverse Primer	CCGCACAAAAAACCGCA	270
	SHOX2_Pb_A5	Flap Oligo.	CCACGGACGATCCGCAAACGC/3C6/	271
ZDHHC1	ZDHHC1FP	Forward Primer	GTCGGGGTCGATAGTTTACG	348
	ZDHHC1RP_V3	Reverse Primer	ACTCGAACTCACGAAAACG	349
	ZDHHC1Probe_v3_A1	Flap Oligo.	CGCCGAGGGACGAACGCACG/3C6/	350
BIN2_Z	BIN2_FP_Z	Forward Primer	GGGTTTATTTTTAGGTAGCGTTCG	50
	BIN2_RP_Z	Reverse Primer	CGAAATTTCGAACAAAAATTAAAACTCGA	51
	BIN2_Pb_A5_Z	Flap Oligo.	CCACGGACGGTTCGAGGTTAG/3C6/	52
SKI	SKI_FP	Forward Primer	ACGGTTTTTTCGTTATTTTTACGGG	279
	SKI_RP	Reverse Primer	CAACGCCTAAAAACACGACTC	280
	SKI_Pb_A1	Flap Oligo.	CGCCGAGGGGCGGTTGTTGG/3C6/	281
DNMT3A	DNMT3A_FP	Forward Primer	GTTACGAATAAAGCGTTGGCG	93
	DNMT3A_RP	Reverse Primer	AACGAAACGTCTTATCGCGA	94
	DNMT3A_Pb_A5	Flap Oligo.	CCACGGACGGAGTGCGCGTTC/3C6/	95
BC2L11	BCL2L11_FP	Forward Primer	CGTAATGTTTCGCGTTTTTCG	35

	BCL2L11_RP	Reverse Primer	ACTTTCTTCTACGTAATTCTTTTCCGA	36
	BCL2L11_Pb_A1	Flap Oligo.	CGCCGAGGGCGGGGTCGGGC/3C6/	37
TBX15	TBX15_Reg2_FP	Forward Primer	AGGAAATTGCGGGTTTTCG	332
	TBX15_Reg2_RP	Reverse Primer	CCAAAAATCGTCGCTAAAAATCAAC	334
	TBX15_Reg2_Pb_A5	Flap Oligo.	CCACGGACGCGCGCATTCACT/3C6/	335
FERMT3	FERMT3_FP	Forward Primer	GTTTTCGGGGATTATATCGATTCG	118
	FERMT3_RP	Reverse Primer	CCCAATAACCCGCAAAATAACC	119
	FERMT3_Pb_A1	Flap Oligo.	CGCCGAGGCGACTCGACCTC/3C6/	120
PRKCB_28	PRKCB_28_FP	Forward Primer	GGAAGGTGTTTTGCGCG	249
	PRKCB_28_RP	Reverse Primer	CTTCTACAACCACTACACCGA	250
	PRKCB_28_Pb_A5	Flap Oligo.	CCACGGACGGCGCGCGTTTAT/3C6/	251
SOBP_HM	SOBP_HM_FP	Forward Primer	TTTCGGCGGGTTTCGAG	294
	SOBP_HM_RP	Reverse Primer	CGTACCGTTCACGATAACGT	295
	SOBP_HM_Pb_A1	Flap Oligo.	CGCCGAGGGGCGGTCGCGGT/3C6/	296
MAX.chr8.145	MAX.Chr8.145_FP	Forward Primer	GCGGTATTAGTTAGAGTTTTAGTCG	211
	MAX.Chr8.145_RP	Reverse Primer	ACAACCCTAAACCCTAAATATCGT	212
	MAX.Chr8.145_Pb_A5	Flap Oligo.	CCACGGACGGACGGCGTTTTT/3C6/	213
MAX.chr10.226	MAX.Chr10.226_FP	Forward Primer	GGGAAATTTGTATTTCGTAAAATCG	178
	MAX.Chr10.226_RP	Reverse Primer	ACAACTAACTTATCTACGTAACATCGT	179
	MAX_Chr10.226_Pb_A1	Flap Oligo.	CGCCGAGGGCGGTTAAGAAA/3C6/	180
MAX.chr12.52	MAX.Chr12.52_FP	Forward Primer	TCGTTCGTTTTTGTCGTTATCG	183
	MAX.Chr12.52_RP	Reverse Primer	AACCGAAATACAACTAAAAACGC	184
	MAX.Chr12.52PbA1	Flap Oligo.	CCACGGACGCGAACCCCGCAA/3C6/	185
FLJ45983	FLJ45983_FP	Forward Primer	GGGCGCGAGTATAGTCG	133
	FLJ45983_RP	Reverse Primer	CAACGCGACTAATCCGC	134
	FLJ45983_Pb_A1	Flap Oligo.	CGCCGAGGCCGTCACCTCCA/3C6/	135
HOXA9	HOXA9_FP	Forward Primer	TTGGGTAATTATTACGTGGATTCG	148
	HOXA9_RP	Reverse Primer	ACTCATCCGCGACGTC	149
	HOXA9_Pb_A5	Flap Oligo.	CCACGGACGCGACGCCCAACA/3C6/	150

EMX1	EMX1_FP	Forward Primer	GGCGTCGCGTTTTTTAGAGAA	108
	EMX1_RP	Reverse Primer	TTCCTTTTCGTTCGTATAAAATTTCGTT	109
	EMX1PbA1	Flap Oligo.	CGCCGAGGATCGGGTTTTAG/3C6/	110
SP9	SP9_FP	Forward Primer	TAGCGTCGAATGGAAGTTCGA	315
	SP9_RP	Reverse Primer	GCGCGTAAACATAACGCACC	317
	SP9_Pb_A5	Flap Oligo.	CCACGGACGCCGTACGAATCC/3C6/	318
DMRTA2	DMRTA2_FP	Forward Primer	TGGTGTTTACGTTCGGTTTTCGT	88
	DMRTA2_RP	Reverse Primer	CCGCAACAACGACGACC	89
	DMRTA2_Pb_A1	Flap Oligo.	CGCCGAGGCGAACGATCACG/3C6/	90
OPLAH	FPrimerOPLAH	Forward Primer	cGTcGcGTTTTTcGGTTATACG	231
	RPrimerOPLAH	Reverse Primer	CGCGAAAACTAAAAAACCGCG	232
	ProbeA5OPLAH	Flap Oligo.	CCACGGACG-GCACCGTAAAAC/3C6/	233
CYP26C1	CYP26C1_FP	Forward Primer	TGGTTTTTTGGTTATTTCGGAATCGT	70
	CYP26C1_RP	Reverse Primer	GCGCGTAATCAACGCTAAC	71
	CYP26C1_Pb_A1	Flap Oligo.	CGCCGAGGCGACGATCTAAC/3C6/	72
ZNF781	ZNF781F.primer	Forward Primer	CGTTTTTTTGTTTTTCGAGTGCG	373
	ZNF781R.primer	Reverse Primer	TCAATAACTAAACTCACCGCGTC	374
	ZNF781probe.A5	Flap Oligo.	CCACGGACGGCGGATTTATCG/3C6/	375
DLX4	DLX4_FP	Forward Primer	TGAGTGCGTAGTGTTTTCGG	80
	DLX4_RP	Reverse Primer	CTCCTCTACTAAAACGTACGATAAACA	81
	DLX4_Pb_A1	Flap Oligo.	CGCCGAGGATCGTATAAAAC/3C6/	82
SUCLG2	SUCLG2_HM_FP	Forward Primer	TCGTGGGTTTTTAATCGTTTCG	321
	SUCLG2_HM_RP	Reverse Primer	TCACGCCATCTTTACCGC	322
	SUCLG2_HM_Pb_A5	Flap Oligo.	CCACGGACGCGAAAATCTACA/3C6/	323
KLHDC7B	KLHDC7B_FP	Forward Primer	AGTTTTCGGGTTTTGGAGTTCGTTA	158
	KLHDC7B_RP	Reverse Primer	CCAAATCCAACCGCCGC	159
	KLHDC7B_Pb_A1	Flap Oligo.	CGCCGAGGACGGCGGTAGTT/3C6/	160
S1PR4_HM	S1PR4_HM_FP	Forward Primer	TTATATAGGCGAGGTTGCGT	284
	S1PR4_HM_RP	Reverse Primer	CTTACGTATAAATAATACAACCACCGAATA	285
	S1PR4_HM_Pb_A5	Flap Oligo.	CCACGGACGACGTACCAAACA/3C6/	286
NFIX_HM	NFIX_HM_FP	Forward Primer	TGGTTCGGGCGTGACGCG	221

	NFIX_HM_RP	Reverse Primer	TCTAACCCTATTTAACCAACCGA	222
	NFIX_HM_Pb_A1	Flap Oligo.	CGCCGAGGGCGGTTAAAGTG/3C6/	223
Reference DNAs	**Oligonucleotide Name**	**Component**	**Sequence (5'-3')**	
Zebrafish Synthetic (RASSF1) BT converted) †	ZF_RASSF1_FP	BT Forward Primer	TGCGTATGGTGGGCGAG	394
	ZF_RASSF1_RP	BT Reverse Primer	CCTAATTTACACGTCAACCAATCGAA	395
	ZF_RASSF1_Pb_A5	BT Flap Oligo.	CCACGGACGGCGCGTGCGTTT/3C6/	397
B3GALT6*	B3GALT6_FP_V2	Forward Primer	GGTTTATTTTGGTTTTTTGAGTTTTCGG	386
	B3GALT6_RP	Reverse Primer	TCCAACCTACTATATTTACGCGAA	387
	B3GALT6_Pb_A1	Flap Oligo.	CCACGGACGGCGGATTTAGGG/3C6/	388
BTACT	ACTB_BT_FP65	Forward Primer	GTGTTTGTTTTTTTGATTAGGTGTTTAAGA	381
	ACTB_BT_RP65	Reverse Primer	CTTTACACCAACCTCATAACCTTATC	382
	ACTBBTPbA3	Flap Oligo.	GACGCGGAGATAGTGTTGTGG/3C6/	383

*The B3GALT6 marker is used as both a cancer methylation marker and as a reference target. See U.S. Pat. Appl. Ser. No. 62/364,082, filed 07/19/16, which is incorporated herein by reference in its entirety.

†For zebrafish reference DNA see U.S. Pat. Appl. Ser. No. 62/364,049, filed 07/19/16, which is incorporated herein by reference in its entirety.

The DNA prepared from plasma as described above was amplified in two multiplexed pre-amplification reactions, as described in Example 1. The multiplex pre-amplification reactions comprised reagents to amplify the following marker combinations.

TABLE 4

Multiplex Mix 1	Multiplex Mix 2
B3GALT6 (reference)	B3GALT6 (reference)
ZF_RASSF1 (reference)	ZF_RASSF1 (reference)
BARX1	CYP26C1
BCL2L11	DLX4
BCL2L11	DMRTA2
BIN2_Z	EMX1
DNMT3A	HOXA9
FERMT3	KLHDC7B
PARP15	MAX.chr8.125

PRKCB_28	MAX_chr10.226
SHOX2	NFIX
SLC12A8	OPLAH
SOBP	S1PR4
TBX15_Reg2	SP9
ZDHHC1	SUCLG2
	ZNF781

Following pre-amplification, aliquots of the pre-amplified mixtures were diluted 1:10 in 10 mM Tris HCl, 0.1 mM EDTA, then were assayed in triplex QuARTS PCR-flap assays, as described in Example 1. The Group 1 triplex reactions used pre-amplified material from Multiplex Mix 1, and the Group 2 reactions used the pre-amplified material from Multiplex Mix 2. The triplex combinations were as follows:

Group 1:

ZF_RASSF1-B3GALT6-BTACT	(ZBA Triplex)
BARX1-SLC12A8-BTACT	(BSA2 Triplex)
PARP15-MAX.chr8.124-BTACT	(PMA Triplex)
SHOX2-ZDHHC1-BTACT	(SZA2 Triplex)
BIN2_Z-SKI-BTACT	(BSA Triplex)
DNMT3A-BCL2L11-BTACT	(DBA Triplex)
TBX15-FERMT3-BTACT	(TFA Triplex)
PRKCB_28-SOBP-BTACT	(PSA2 Triplex)

Group 2:

ZF_RASSF1-B3GALT6-BTACT	(ZBA Triplex)
MAX.chr8.145-MAX_chr10.226-BTACT	(MMA2 Triplex)
MAX.chr12.526-FLJ45983-BTACT	(MFA Triplex)
HOXA9-EMX1-BTACT	(HEA Triplex)
SP9-DMRTA2-BTACT	(SDA Triplex)
OPLAH-CYP26C1-BTACT	(OCA Triplex)
ZNF781-DLX4-BTACT	(ZDA Triplex)
SUCLG2-KLHDC7B-BTACT	(SKA Triplex)
S1PR4-NFIX-BTACT	(SNA Triplex)

Each triplex acronym uses the first letter of each gene name (for example, the combination of HOXA9-EMX1-BTACT = "HEA"). If an acronym is repeated for a different combination of markers or from another experiment, the second grouping having that acronym includes the number 2. The dye reporters used on the FRET cassettes for each member of the triplexes listed above is FAM-HEX-Quasar670, respectively.

Plasmids containing target DNA sequences were used to calibrate the quantitative reactions. For each calibrator plasmid, a series of 10X calibrator dilution stocks, having from 10 to 10^6 copies of the target strand per µl in fish DNA diluent (20 ng/mL fish DNA in 10 mM Tris-HCl, 0.1 mM EDTA) were prepared. For triplex reactions, a combined stock having plasmids that contain each of the targets of the triplex were used. A mixture having each plasmid at $1x10^5$ copies per µL was prepared and used to create a 1:10 dilution series. Strands in unknown samples were back calculated using standard curves generated by plotting Cp vs Log (strands of plasmid).

Using receiver operating characteristic (ROC) curve analysis, the area under the curve (AUC) for each marker was calculated and is shown in the table below, sorted by Upper 95 Pct Coverage Interval.

TABLE 5

Marker Name	AUC	Sensitivity at 90% specificity
CYP26C1	0.940	80%
SOBP	0.929	80%
SHOX2	0.905	73%
SUCLG2	0.905	64%
NFIX	0.895	63%
ZDHHC1	0.890	69%
BIN2_Z	0.872	59%
DLX4	0.856	56%
FLJ45983	0.834	67%
HOXA9	0.824	53%
TBX15	0.813	53%
ACTB	0.803	50%
S1PR4	0.802	55%
SP9	0.782	38%

Marker Name	AUC	Sensitivity at 90% specificity
FERMT3	0.773	36%
ZNF781	0.769	55%
B3GALT6	0.746	39%
BTACT	0.742	44%
BCL2L11	0.732	39%
PARP15	0.673	31%
DNMT3A	0.689	20%
MAX.chr12.526	0.668	33%
MAX.chr10.226	0.671	30%
SLC12A8	0.655	19%
BARX1	0.663	25%
KLHDC7B	0.604	10%
OPLAH	0.571	14%
MAX.chr8.145	0.572	16%
SKI	0.521	14%

The markers worked very well in distinguishing samples from cancer patients from samples from normal subjects (see ROC table, above). Use of the markers in combination improved sensitivity. For example, using a logistic fit of the data and a six-marker fit, ROC curve analysis shows an AUC = 0.973.

Using a 6-marker fit, sensitivity of 92.2% is obtained at 93% specificity. The group of 6 markers that together resulted in the best fit was *SHOX2, SOBP, ZNF781, BTACT, CYP26C1,* and *DLX4* (see Fig 7). Using *SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2,* and *SKI gave an ROC* curve with AUC of 0.97982 (see Fig. 8).

EXAMPLE 4

Archival plasmas from a second independent study group were tested in blinded fashion. Lung cancer cases and controls (apparently healthy smokers) for each group were balanced on age and sex (23 cases, 80 controls). Using multiplex PCR followed by QuARTS (Quantitative Allele-Specific Real-time Target and Signal amplification) assay as described in Example 1, a post-bisulfite quantification of methylated DNA markers on DNA extracted from plasma was performed. Top individual methylation markers from Example 3 were

tested in this experiment to identify optimal marker panels for lung cancer detection (2 ml/patient).

Results: 13 high performance methylated DNA markers were tested (*CYP26C1, SOBP, SUCLG2, SHOX2, ZDHHC1, NFIX, FLJ45983, HOXA9, B3GALT6, ZNF781, SP9, BARX1,* and *EMX1*). Data were analyzed using two methods: a logistic regression fit and a regression partition tree approach. The logistic fit model identified a 4-marker panel (*ZNF781, BARX1, EMX1,* and *SOBP*) with an AUC of 0.96 and an overall sensitivity of 91% and 90% specificity. Analysis of the data using a regression partition tree approach identified 4 markers (*ZNF781, BARX1, EMX1,* and *HOXA9*) with AUC of 0.96 and an overall sensitivity of 96% and specificity of 94%. For both approaches, B3GALT6 was used as a standardizing marker of total DNA input. These panels of methylated DNA markers assayed in plasma achieved high sensitivity and specificity for all types of lung cancer.

EXAMPLE 5

Differentiating Lung Cancers

Using the methods described above, methylation markers are selected that exhibit high performance in detecting methylation associated with specific types of lung cancer.

For a subject suspected of having lung cancer, a sample is collected, *e.g.*, a plasma sample, and DNA is isolated from the sample and treated with bisulfite reagent, *e.g.*, as described in Example 1. The converted DNA is analyzed using a multiplex PCR followed by QuARTS flap endonuclease assay as described in Example 1, configured to provide different identifiable signals for different methylation markers or combinations of methylation markers, thereby providing data sets configured to specifically identify the presence of one or more different types of lung carcinoma in the subject (*e.g.*, adenocarcinoma, large cell carcinoma, squamous cell carcinoma, and/or small cell carcinoma). In preferred embodiments, a report is generated indicating the presence or absence of an assay result indicative of the presence of lung carcinoma and, if present, further indicative of the presence of one or more identified types of lung carcinoma. In some embodiments, samples from a subject are collected over the course of a period of time or a course of treatment, and assay results are compared to monitor changes in the cancer pathology.

Marker and marker panels sensitive to different types of lung cancer find use, *e.g.*, in classifying type(s) of cancer present, identifying mixed pathologies, and/or in monitoring cancer progression over time and/or in response to treatment.

EXAMPLE 6

Using multiplex PCR followed by QuARTS (Quantitative Allele-Specific Real-time Target and Signal amplification) assay as described in Example 1, a post-bisulfite quantification of methylated DNA markers on DNA extracted from plasma was performed. The target sequences, bisulfite converted target sequences, and the assay oligonucleotides for these markers were as shown in Fig. 1. The primers and flap oligonucleotides (probes) used for each converted target were as follows:

TABLE 6

Marker	Oligonucleotide Name	Component	Sequence (5'-3')	SEQ ID NO:	Arm
BARX1	BARX1_FP	Primer	CGTTAATTTGTTAGATAGAGGGCG	23	5-FAM
	BARX1_RP_universal	Primer	TCCGAACAACCGCCTAC	26	
	BARX1_Pb_A5_63_v6	Flap Oligo.	**AGGCCACGGACG** CGAAAAATCCCACGC/3C6/	405	
FLJ45983	FLJ45983_FP_v4	Primer	CGAGGTTATGGAGGTGACG	409	5-FAM
	FLJ45983_RP_v4	Primer	CGAATACTACCCGTTAAACACG	410	
	FLJ45983_Pb_A5_63_v4	Flap Oligo.	**AGGCCACGGACG** GGCGGATTAGTCGCG/3C6/	411	
HOXA9	HOXA9_FP	Primer	TTGGGTAATTATTACGTGGATTCG	148	5-FAM
	HOXA9_RP_v2	Primer	CAACTCATCCGCGACG	423	
	HOXA9_Pb_A5_63	Flap Oligo.	**AGGCCACGGACG** GTCGACGCCCAACAA/3C6/	424	
HOPX	HOPX_2149_FP	Primer	GTAGCGCGTAGGGATTATGTCG	417	5-FAM
	HOPX_2149_RP	Primer	TTTCCACCTAATCCTCTATAAAACCGC	418	
	HOPX_2149_Pb_A5	Flap Oligo.	**AGGCCACGGACG** CTCGCGATCTCCGC/3C6/	419	
ZNF781	ZNF781 F.primer	Primer	CGTTTTTTTGTTTTTCGAGTGCG	373	5-FAM
	ZNF781	Primer	TCAATAACTAAACTCACCGCGTC	374	

	R.primer				
	ZNF781_Pb_A5_63_v2	Flap Oligo.	**AGGCCACGGACG** GCGGATTTATCGGGTTATAGT/3C6/	435	
HOXB2	HOXB2_FP	Primer	GTTAGAAGACGTTTTTTCGGGG	153	1-HEX
	HOXB2_RP	Primer	AAAACAAAAATCGACCGCGA	154	
	HOXB2_Pb_A1_63	Flap Oligo.	**CGCGCCGAGG** GCGTTAGGATTTATTTTTTTTTTCGA/3C6/	425	
IFFO1	IFFO1_FP_HQ_corrected	Primer	CGGGATAGAGTCGATTAATTAGGC	428	1-HEX
	IFFO1_RP	Primer	TAACTTCCCCTCGACCCG	429	
	IFFO1_Pb_A1_63	Flap Oligo.	**CGCGCCGAGG** CGGTTCGGTAGCGG/3C6/	430	
SOBP	SOBP HM FP	Primer	TTTCGGCGGGTTTCGAG	294	1-HEX
	SOBP HM RP	Primer	CGTACCGTTCACGATAACGT	295	
	SOBP HM Pb A1 63	Flap Oligo.	**CGCGCCGAGG** TTACAAACCGCGACCG/3C6/	431	
TRH	TRH_FP	Primer	TTTTCGTTGATTTTATTCGAGTCGTC	432	1-HEX
	TRH_RP	Primer	GAACCCTCTTCAAATAAACCGC	433	
	TRH_Pb_A1_63	Flap Oligo.	**CGCGCCGAGG** CGTTTGGCGTAGATATAAGC/3C6/	434	
FAM59B	FAM59B_FP_V3	Primer	GTCGAGCGTTTGGTGCG	406	1-HEX
	FAM59B_RP_V3	Primer	CTCGTCGAAATCGAAACGC	407	
	FAM59B_Pb_A1_63_V3	Flap Oligo.	**CGCGCCGAGG** GCGATAGCGTTTTTTATTGTCG/3C6/	408	

*All methylation assays were triplexed with an assay for bisulfite-converted B3GALT6 marker, reporting to Quasar:

Marker	Oligonucleotide Name	Component	Sequence (5'-3')	SEQ ID NO:	
B3GALT6 (BST)	B3GALT6_FP_V2	Primer	GGTTTATTTTGGTTTTTTGAGTTTTCGG	386	3-Quasar
	B3GALT6_RP	Primer	TCCAACCTACTATATTTACGCGAA	387	
	B3GALT6_Pb_A3_63	Flap Oligo.	ACGGACGCGGAG GCGGATTTAGGGTATTTAAGGAG/3C6/	436	

The DNA prepared from plasma as described above was amplified in a multiplexed pre-amplification reaction, as described in Example 1. Following pre-amplification, aliquots of

the pre-amplified mixtures were diluted 1:10 in 10 mM Tris HCl, 0.1 mM EDTA, then were assayed in triplex QuARTS PCR-flap assays, as described in Example 1. The triplex combinations were as follows:

Triplex Assays
BARX1/HOXB2/B3GALT6 (BHB)
FLJ45983/IFFO1/B3GALT6 (FIB)
HOXA9/SOBP/B3GALT6 (HSB)
HOPX 2149/TRH/B3GALT6 (HTB)
ZNF781/FAM59B/B3GALT6 (ZFB)

Plasmids containing target DNA sequences were used to calibrate the quantitative reactions. For each calibrator plasmid, a series of 10X calibrator dilution stocks, having from 10 to 10^6 copies of the target strand per µl in fish DNA diluent (20 ng/mL fish DNA in 10 mM Tris-HCl, 0.1 mM EDTA) were prepared. For triplex reactions, a combined stock having plasmids that contain each of the targets of the triplex were used. A mixture having each plasmid at $1x10^5$ copies per µL was prepared and used to create a 1:10 dilution series. Strands in unknown samples were back calculated using standard curves generated by plotting Cp vs Log (strands of plasmid).

Individual Marker ROC using % methylation relative to *B3GALT6* strands is shown in Fig 9A to 9I. An ROC analysis for the combination of markers Figure 10 provides a graph showing a 6-marker logistic fit using markers *BARX1, FLJ45983, SOBP, HOPX, IFFO1,* and *ZNF781.* The ROC curve analysis shows an area under the curve (AUC) of 0.85881. Use of the markers in combination improved sensitivity.

EXAMPLE 7

Combination of mRNA and methylation markers to improve lung cancer detection sensitivity

Expression level of *FPR1* mRNA (Formyl Peptide Receptor 1) has been shown previously to be a lung cancer marker detectable in blood (Morris, S., *et al., Int J Cancer.*, (2018) 142:2355-2362). In some embodiments, the methylation marker assays described above are used in combination with measurement of one or more expression markers. An

exemplary combination assay comprises measurement of FPR1 mRNA levels and detection of methylation marker DNA(s) (*e.g.*, as described in Examples 1-6) in a sample or samples from the same subject.

The FPR1 sequence (NM_001193306.1 Homo sapiens formyl peptide receptor 1 (FPR1), transcript variant 1, mRNA, is shown in SEQ ID NO:437. As described by Morris, *et al., supra*, blood samples are collected in a blood collection tube suitable for subsequent RNA detection (*e.g.*, PAXgene Blood RNA Tube; Qiagen, Inc.) Samples may be assayed immediately or frozen until future analysis. RNA is extracted from a sample by standard methods, *e.g.*, Qiasymphony PAXgene blood RNA kit. Levels of RNA, *e.g.*, an mRNA marker, are determined using a suitable assay for measurement of specific RNAs present in a sample, *e.g.*, RT-PCR. In some embodiments, a QuARTS flap endonuclease assay reaction comprising a reverse transcription step is used. See, *e.g.*, U.S. Pat. Appl. No. 15/587,806, which is incorporated herein by reference. In preferred embodiments, assay probes and/or primers for an RT-PCR or an RT-QuARTS assay are designed to span an exon junction(s) so that the assay will specifically detect mRNA targets rather than detecting the corresponding genomic loci.

An exemplary RT-QuARTS reaction contains 20U of MMLV reverse transcriptase (MMLV-RT), 219 ng of Cleavase® 2.0, 1.5U of GoTaq® DNA Polymerase, 200nM of each primer, 500nM each of probe and FRET oligonucleotides, 10mM MOPS buffer, pH7.5, 7.5mM $MgCl_2$, and 250µM each dNTP. Reactions are typically run on a thermal cycler configured to collect fluorescence data in real time (*e.g.*, continuously, or at the same point in some or all cycles). For example, a Roche LightCycler 480 system may be used under the following conditions: 42°C for 30 minutes (RT reaction), 95°C for 3 min, 10 cycles of 95°C for 20 seconds, 63°C for 30 sec, 70°C for 30 sec, followed by 35 cycles of 95°C for 20 sec, 53°C for 1 min, 70°C for 30 sec, and hold at 40°C for 30 sec.

In some embodiments, RT-QuARTS assays may comprise a step of multiplex pre-amplification, *e.g.*, to pre-amplify 2, 5, 10, 12, or more targets in a sample (or any number of targets greater than 1 target), as described above in Example 1. In preferred embodiments, an RT- pre-amplification is conducted in a reaction mixture containing, *e.g.*, 20U of MMLV reverse transcriptase, 1.5U of GoTaq® DNA Polymerase,10mM MOPS buffer, pH7.5, 7.5mM $MgCl_2$, 250µM each dNTP, and oligonucleotide primers, (*e.g.*, for 12 targets, 12 primer pairs/24 primers, in equimolar amounts (*e.g.*, 200nM each primer), or with individual

primer concentrations adjusted to balance amplification efficiencies of the different targets). Thermal cycling times and temperatures are selected to be appropriate for the volume of the reaction and the amplification vessel. For example, the reactions may be cycled as follows:

Stage	Temp / Time	#of Cycles
RT	42°C /30'	1
	95°C / 3'	1
Amplification 1	95°C / 20"	10
	63°C / 30"	
	70°C / 30"	
Cooling	4°C / Hold	1

After thermal cycling, aliquots of the pre-amplification reaction (*e.g.*, 10 µL) are diluted to 500 µL in 10 mM Tris, 0.1 mM EDTA, with or without fish DNA. Aliquots of the diluted pre-amplified DNA (*e.g.*, 10 µL) are used in QuARTS PCR-flap assays, as described above.

In some embodiments, DNA targets, *e.g.*, methylated DNA marker genes, mutation marker genes, and/or genes corresponding to the RNA marker, *etc.*, may be amplified and detected along with the reverse-transcribed cDNAs in a QuARTS assay reaction, *e.g.*, as described in Example 1, above. In some embodiments, DNA and cDNA are co-amplified and detected in a single-tube reaction, *i.e.*, without the need to open the reaction vessel at any point between combining the reagents and collecting the output data. In other embodiments, marker DNA from the same sample or from a different sample may be separately isolated, with or without a bisulfite conversion step, and may be combined with sample RNA in an RT-QuARTS assay. In yet other embodiments, RNA and/or DNA samples may be pre-amplified as described above.

In Morris, ROC curve analysis of the *FPR1* mRNA ratio relative to a housekeeping gene (HNRNPA1) resulted in a sensitivity of 68% at a specificity of 89%, and ROC curve analysis using methylation markers *BARX1, FAM59B, HOXA9, SOBP,* and *IFFO1*, as shown in Fig. 11B, results in a sensitivity of 77.2% at a specificity of 92.3%. Using these assays together results in a theoretical sensitivity of 92.7% at a specificity of 82%.

This analysis shows that a combination assay for levels of *FPR1* mRNA along with detection of one or more methylation markers results in an assay having improved sensitivity compared to either method alone. A cancer detection assay that combines different classes of markers has the advantage of being able to detect the biological differences between early and late diseases stages as well as different biological responses or sources of cancer. It will be clear to one skilled in the art that other RNA targets, including mRNA targets other than or in addition to *FPR1*, such as *LunX* mRNA (Yu, et al., 2014, Chin J Cancer Res., 26:89-94), can be combined with methylation markers for enhanced sensitivity.

EXAMPLE 8

Combination of a protein (*e.g.*, autoantibody) and methylation markers to improve lung cancer detection sensitivity

Tumor-associated antigens in lung and other solid tumors can provoke a humoral immune response in the form of autoantibodies, and these antibodies have been observed to be present very early in the disease course, *e.g.*, prior to the presentation of symptoms. (see Chapman CJ, Murray A, McElveen JE, *et al. Thorax* 2008;**63:**228-233, which is incorporated herein by reference in its entirety for all purposes). However, the sensitivity of autoantibody detection for detecting lung carcinomas is relatively low. For example, autoantibodies to tumor antigen NY-ESO-1 (Accession # P78358, sequence shown as SEQ ID NO: 442; also known as CTAG1B) has been shown in the literature to be a good marker for non small-cell lung cancer (NSCLC; Chapman, *supra*), but it is not sufficiently sensitive to be useful alone. The detection of one or more tumor-associated autoantibodies in combination with the detection of one or more methylation markers provides an assay with greater sensitivity.

Blood samples are collected, and autoantibodies are detected using standard methods, *e.g.*, ELISA detection, as described by Chapman, *supra.* Detecting methylation and/or mutation markers in DNA isolated the samples is done as described in Example 1, above.

Detection of NY-ESO-1 autoantibody alone results in a sensitivity of 40% at 95% specificity (Türeci, *et al.*, Cancer Letters 236(1):64 (2006). As discussed above, the assaying the methylation of the combination of *BARX1, FAM59B, HOXA9, SOBP,* and *IFFO1* markers results in a sensitivity of 77.2% at 92.3% specificity. Combining analysis of this autoantibody marker with the assay for this combination of methylation markers results in a combined theoretical sensitivity of 86.3%, with at specificity of 87.7%.

This analysis shows that combined assays of levels of autoantibodies with analysis of one or more methylation markers results in an assay having improved sensitivity compared to either method alone. A cancer detection assay that combines different classes of markers has the advantage of being able to detect the biological differences between early and late diseases stages as well as different biological responses or sources of cancer.

EXAMPLE 9

Combination of mRNA , methylation marker(s), and protein (*e.g.*, autoantibody) to improve lung cancer detection sensitivity

Analysis of combinations of one or more RNAs, marker DNAs, and autoantibodies in a sample or samples from a subject may be performed for enhanced detection of lung and other cancers in the subject. Methods for sample preparation and DNA, RNA, and protein detection are as discussed above.

As discussed in Example 7, analysis of the *FPR1* mRNA ratio relative to a housekeeping gene (*HNRNPA1*) as reported by Morris, et al. resulted in a sensitivity of 68% at a specificity of 89% (Morris, *supra*); detection of NY-ESO-1 autoantibody alone as reported by Chapman resulted in a sensitivity of 40% at 95% specificity; and assaying the methylation of the combination of *BARX1, FAM59B, HOXA9, SOBP,* and *IFFO1* markers results in a sensitivity of 77.2% at 92.3% specificity. Combining analysis of the mRNA, the autoantibody marker, and the assay for this combination of methylation markers results in a combined theoretical sensitivity of 95.6%, with a specificity of 77.9%, showing that combined assays of levels of mRNA and levels of autoantibodies with analysis of one or more methylation markers results in an assay having improved sensitivity compared to any one of these methods alone.

Assays as described above may be further enhanced by the addition of an assay to detect one or more antigens. Those of skill in the art will appreciate that detection of an antigen may be added to the detection of any of: RNA(s), methylation marker gene(s), and/or autoantibody(ies), individually or in any combination, and will further enhance overall sensitivity.

All literature and similar materials cited in this application, including but not limited to, patents, patent applications, articles, books, treatises, and internet web pages are expressly

incorporated by reference in their entirety for any purpose. Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which the various embodiments described herein belongs. When definitions of terms in incorporated references appear to differ from the definitions provided in the present teachings, the definition provided in the present teachings shall control.

Various modifications and variations of the described compositions, methods, and uses of the technology will be apparent to those skilled in the art without departing from the scope and spirit of the technology as described. Although the technology has been described in connection with specific exemplary 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 pharmacology, biochemistry, medical science, or related fields are intended to be within the scope of the following claims.

CLAIMS

What is claimed is:

1. A method of characterizing a sample, comprising:

a) measuring an amount of at least one methylation marker gene in DNA from the sample, wherein the at least one methylation marker gene comprises at least one of *IFFO1* and *HOPX*;

b) measuring the amount of at least one reference marker in the DNA; and

c) calculating a value for the amount of the at least one methylation marker measured in the DNA as a percentage of the amount of the reference marker measured in the DNA, wherein the value indicates the amount of the at least one methylation marker gene measured in the sample.

2. The method of claim 1, wherein said at least one methylation marker gene consists of one to fifteen methylation marker genes.

3. The method of claim 1, wherein the at least one methylation marker gene comprises one or more marker genes selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1* and *ZNF32.*

4. The method of claim 1, wherein the at least one methylation marker gene consists of at least one of *IFFO1* and *HOPX,* and further comprises one or more of *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B.*

5. The method of claim 4, wherein the at least one methylation marker gene consists of:

at least one of *IFFO1* and *HOPX*; and

the group consisting of *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B.*

6. The method of any one of claims 1 to 5, wherein the at least one reference marker comprises one or more reference marker selected from *B3GALT6* DNA and β-actin DNA.

7. The method of any one of claims 1 to 6, wherein the DNA is treated with a reagent that selectively modifies DNA in a manner specific to the methylation status of the DNA.

8. The method of claim 7, wherein the reagent comprises a bisulfite reagent, a methylation-sensitive restriction enzyme, or a methylation-dependent restriction enzyme.

9. The method of any one of claims 1 to 8, wherein the sample comprises one or more of tissue, blood, serum, plasma, and sputum.

10. The method of any one of claims 1 to 9, wherein the DNA is extracted from the sample.

11. The method of any one of claims 1 to 10, wherein the DNA is treated with a bisulfite reagent to produce bisulfite-treated DNA.

12. The method of any one of claims 1 to 11 wherein measuring amounts of a methylation marker gene comprises using one or more of polymerase chain reaction, nucleic acid sequencing, mass spectrometry, methylation-specific nuclease, mass-based separation, and target capture.

13. The method of claim 12, wherein the measuring comprises multiplex amplification.

14. The method of any one of claims 1 to 13, wherein measuring the amount of at least one methylation marker gene comprises using one or more methods selected from the group consisting of methylation-specific PCR, quantitative methylation-specific PCR, methylation-

specific DNA restriction enzyme analysis, quantitative bisulfite pyrosequencing, flap endonuclease assay, PCR-flap assay, and bisulfite genomic sequencing PCR.

15. A method of characterizing at least one sample from a subject, comprising

a) measuring an amount of at least one methylation marker gene in DNA from a sample obtained from a subject, the method comprising:

i) measuring an amount of at least one reference marker in the DNA; and

iii) calculating a value for the amount of the at least one methylation marker gene measured in the DNA as a percentage of the amount of the reference marker measured in the DNA, wherein the value indicates the amount of the at least one methylation marker gene measured in the sample;

and one or more of

b) measuring an amount of at least one RNA marker in a sample obtained from the subject;

and

c) assaying for the presence or absence of at least one protein marker in a sample obtained from the subject.

16. The method of claim 15, wherein measuring an amount of at least one RNA marker in a sample comprises:

i) measuring an amount of a reference RNA in the sample; and

ii) calculating a value for the amount of the at least one RNA marker measured in the sample as a percentage of the amount of reference RNA measured in the sample, wherein the value indicates the amount of the at least one RNA marker measured in the sample, wherein the amount of the at least one RNA marker in the sample is indicative of a level of expression for a gene for said at least one RNA marker.

17. The method of claim 15 or claim 16, wherein the at least one RNA marker comprises mRNA.

18. The method of claim 17, wherein the at least one RNA marker comprises mRNA selected from the group consisting of *GAGE12D, FAM83A, LRG1, XAGE-1 d, MAGEA4, SFTPB, AKAP4,* and *CYP24A1.*

19. The method of any one of claims 15 to 18, wherein said reference RNA is selected from the group consisting of *CASC3* mRNA, *β-actin* mRNA, *U1* snRNA and *U6* snRNA.

20. The method of any one of claims 15 to 19, wherein the at least one methylation marker gene comprises one or more marker genes selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, ZNF32, IFFO1* and *HOPX.*

21. The method of any one of claims 15 to 20, wherein the protein is an autoantibody.

22. The method of claim 21, wherein the autoantibody is an antibody to a cancer-associated antigen.

23. The method any one of claims 15 to 20, wherein the protein is a cancer-associated antigen.

24. The method of any one of claims 15 to 23, comprising measuring an amount of a methylation marker gene, measuring an amount of an RNA, and assaying for the presence or absence of a protein.

25. The method of any one of claims 15 to 24, wherein said measuring and assaying are conducted on a single sample from the subject.

26. A kit, comprising:

a) at least one marker oligonucleotide, wherein at least a portion of said oligonucleotide specifically hybridizes to a methylation marker selected from the group consisting of *IFFO1* and *HOPX,* and

b) at least one reference oligonucleotide, wherein at least a portion of said reference oligonucleotide specifically hybridizes to a reference nucleic acid.

27. The kit of claim 26, further comprising one or more additional marker oligonucleotides, wherein each of the one or more additional marker oligonucleotides specifically hybridizes to a methylation marker gene selected from the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12A8, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1* and *ZNF32.*

28. The kit of any one of claims 26 to 27, wherein said portion of said marker oligonucleotide specifically hybridizes to a bisulfite-treated DNA comprising said methylation marker.

29. The kit of any one of claims 26 to 28, wherein said kit comprises at least two additional marker oligonucleotides.

30. The kit of any one of claims 26 to 29, wherein said kit further comprises one or more of a methylation-specific restriction enzyme and a bisulfite reagent.

31. The kit of any one of claims 26 to 30, wherein said at least one methylation marker comprises at least one of *IFFO1* and *HOPX*, and further comprises one or more methylation

markers selected from the group consisting of *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B.*

32. The kit of any one of claims 26 to 31, wherein said at least one methylation marker consists of:

at least one of *IFFO1* and *HOPX*; and

the group consisting of *BARX1, FLJ45983, HOXA9, ZNF781, HOXB2, SOBP, TRH,* and *FAM59B.*

33. The kit of any one of claims 26 to 32, wherein said at least one marker oligonucleotide is selected from one or more of a capture oligonucleotide, a pair of nucleic acid primers, a nucleic acid probe, and an invasive oligonucleotide.

34. The kit of any one of claims 26 to 33, wherein said kit further comprises a solid support.

35. The kit of claim 34, wherein said solid support is a magnetic bead.

36. The kit of claim 34 or 35, wherein said solid support comprises one or more capture reagents.

37. The kit of claim 36, wherein said capture reagents are oligonucleotides complementary said one or more methylation markers.

38. A composition comprising a reaction mixture comprising at least one complex comprising a methylation marker DNA and a marker oligonucleotide specifically hybridized to the methylation marker DNA, wherein the methylation marker DNA is selected from *IFFO1* and *HOPX,* and an additional complex comprising an additional methylation marker DNA and an additional marker oligonucleotide specifically hybridized to the additional methylation marker DNA, wherein the additional methylation marker DNA is selected from said the group consisting of *BARX1, LOC100129726, SPOCK2, TSC22D4, MAX.chr8.124, RASSF1, ZNF671, ST8SIA1, NKX6_2, FAM59B, DIDO1, MAX_Chr1.110, AGRN, SOBP, MAX_chr10.226, ZMIZ1, MAX_chr8.145, MAX_chr10.225, PRDM14, ANGPT1, MAX.chr16.50, PTGDR_9, ANKRD13B, DOCK2, MAX_chr19.163, ZNF132, MAX*

chr19.372, HOXA9, TRH, SP9, DMRTA2, ARHGEF4, CYP26C1, ZNF781, PTGDR, GRIN2D, MATK, BCAT1, PRKCB_28, ST8SIA_22, FLJ45983, DLX4, SHOX2, EMX1, HOXB2, MAX.chr12.526, BCL2L11, OPLAH, PARP15, KLHDC7B, SLC12a, BHLHE23, CAPN2, FGF14, FLJ34208, B3GALT6, BIN2_Z, DNMT3A, FERMT3, NFIX, S1PR4, SKI, SUCLG2, TBX15, ZDHHC1, and *ZNF32.*

39. The composition of claim 38, wherein said methylation marker DNAs are bisulfite-converted methylation marker DNA.

40. The composition of claim 38 or claim 39, wherein said marker oligonucleotides comprise one or more of a capture oligonucleotide, a pair of nucleic acid primers, a hybridization probe, a hydrolysis probe, a flap assay probe, and an invasive oligonucleotide.

41. The composition of any one of claims 38 or 40, comprising a methylation marker DNA comprising a nucleic acid sequence selected from SEQ ID NOS: 412 and 426 and complements thereof, wherein the additional methylation marker DNA comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: 1, 6, 11, 16, 21, 28, 33, 38, 43, 48, 53, 58, 63, 68, 73, 78, 86, 91, 96, 101, 106, 111, 116, 121, 126, 131, 136, 141, 146, 151, 156, 161, 166, 171, 176, 181, 186, 191, 196, 201, 214, 219, 224, 229, 234, 239, 247, 252, 257, 262, 267, 272, 277, 282, 287, 292, 298, 303, 308, 313, 319, 327, 336, 341, 346, 351, 356, 361, 366, 371, 384, and 403, and complements thereof.

42. The composition of any one of claims 39 to 40, comprising a methylation marker DNA comprising a nucleic acid sequence selected from SEQ ID NOS: 413 and 427 and complements thereof, wherein the additional methylation marker DNA comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOS: SEQ ID NOS: 2, 7, 12, 17, 22, 29, 34, 39, 44, 49, 54, 59, 64, 69, 74, 79, 87, 92, 97, 102, 107, 112, 117, 122, 127, 132, 137, 142, 147, 152, 157, 162, 167, 172, 177, 182, 187, 192, 197, 202, 210, 215, 220, 225, 230, 235, 240, 248, 253, 258, 263, 268, 273, 278, 283, 288, 293, 299, 304, 309, 314, 320, 328, 337, 342, 347, 352, 357, 362, 367, 372, 385, and 404, and complements thereof.

43. The composition of any one of claims 38 to 42, wherein each of said marker oligonucleotides comprises a reporter molecule.

44. The composition of claim 43, where said reporter molecule comprises a fluorophore.

45. The composition of claim any one of claims 38 to 44, wherein one or more of said marker oligonucleotides comprises a flap sequence.

46. The composition of any one of claims 38 to 45, further comprising one or more of a FRET cassette; a FEN-1 endonuclease and a thermostable DNA polymerase.

FIG. 1

AGRN Target DNA (SEQ ID NO:1)

5′GTTCCCGGAACGGCCTCTTGGGGGCGTTCCAGCCCCACGGACCCGCAGGGAGTCCCCGCCGCAATTTGCATGGGG
CTCATTTGCATGACCCCGCCCCGCGCGGGAGTCGGGGGCGC3′

Bisulfite-converted Target DNA: (SEQ ID NO:2)

5′GTTTTCGGAACGGTTTTTTGGGGGCGTTTTAGTTTTACGGATTCGTAGGGAGTTTTCGTCGTAATTTGTATGGGG
TTTATTTGTATGATTTCGTTTCGCGCGGGAGTCGGGGGCGT3′

PCR and Flap Assay Oligonucleotides:

AGRN Forward Primer:	5′ GGCGTTTTAGTTTTACGGATTCG3′ (SEQ ID NO:3)
AGRN Reverse Primer:	5′ ACAAATAAACCCCATACAAATTACGAC3′ (SEQ ID NO:4)
AGRN Flap oligo.:	5′ CGCCGAGGCGAAAACTCCCT/3C6/ (SEQ ID NO:5)

FIG. 1 (cont'd)

ANGPT1 Target DNA: (SEQ ID NO:6)

5'CGGATTCAACATGGGCAATGTGCCTACACTTTCATTCTTCCAGAACACGATGGCAACTGTCGTGAGAGTACGACA
GACCAGTACAACACAAACGCTCTGCAGAGAGATGCTCCACACGTGGAACCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:7)

5'CGGATTTAATATGGGTAATGTGTTTATATTTTTATTTTTTTAGAATACGATGGTAATTGTCGTGAGAGTACGATA
GATTAGTATAATATAAACGTTTTGTAGAGAGATGTTTTATACGTGGAATCG3'

PCR and Flap Assay Oligonucleotides:

ANGPT1 Forward Primer: 5'TTTTAGAATACGATGGTAATTGTCGT3' (SEQ ID NO:8)
ANGPT1 Reverse Primer: 5'ACATCTCTCTACAAAACGTTTATATTATACTAATC3'(SEQ ID NO:9)
ANGPT1 Flap oligo.: 5'CGCCGAGGCTATCGTACTCT/3C6/ (SEQ ID NO:10)

FIG. 1 (cont'd)

ANKRD13B Target DNA: (SEQ ID NO:11)

5'GGAGCTACGACGAGCAGCTGCGGCTGGCGATGGAACTGTCGGCGCAGGAGCAGGAGGAGAGGCGGCGGCGCGCGC
GCCAGGAGGAGGAGGAGCTGGAGCGCATCCTGAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:12)

5'GGAGTTACGACGAGTAGTTGCGGTTGGCGATGGAATTGTCGGCGTAGGAGTAGGAGGAGAGGCGGCGGCGCGCGC
GTTAGGAGGAGGAGGAGTTGGAGCGTATTTTGAG3'

PCR and Flap Assay Oligonucleotides:

ANKRD13B Forward Primer: 5'AGTTACGACGAGTAGTTGCG3'(SEQ ID NO:13)
ANKRD13B Reverse Primer: 5'TCCTCCTACTCCTACGCC3'(SEQ ID NO:14)
ANKRD13B Flap oligo.: 5'CCACGGACGCGACAATTCCAT/3C6/(SEQ ID NO:15)

FIG. 1 (cont'd)

ARHGEF4 Target DNA: (SEQ ID NO:16)

5′GGTGGCAACGGCTGGAGTGCCGTCGCCCGCGCCACTCACCCCGGCGCGGCGCCCTGCGCGGCCGCTCAGCGGAAG
GCCAGCAGGAAGATCAGTACGACGTTGATGAGAACCAGGAGCGCCAGCACGGCGGAGACCACCACGCG3′

Bisulfite-converted Target DNA: (SEQ ID NO:17)

5′GGTGGTAACGGTTGGAGTGTCGTCGTTCGCGTTATTTATTTCGGCGCGGCGTTTTGCGCGGTCGTTTAGCGGAAG
GTTAGTAGGAAGATTAGTACGACGTTGATGAGAATTAGGAGCGTTAGTACGGCGGAGATTATTACGCG3′

PCR and Flap Assay Oligonucleotides:

ARHGEF4 Forward Primer: 5′CGTTCGCGTTATTTATTTCGGCG3′ (SEQ ID NO:18)
ARHGEF4 Reverse Primer: 5′GCTCCTAATTCTCATCAACGTCGT3′ (SEQ ID NO:19)
ARHGEF4 Flap oligo.: 5′CGCCGAGGGCGGCGTTTTGC/3C6/ (SEQ ID NO:20)

FIG. 1 (cont'd)

B3GALT6 Target DNA: (SEQ ID NO:384)

5′GGCCACACAGGCCCACTCTGGCCCTCTGAGCCCCCGGCGGACCCAGGGCATTCAAGGAGCGGCTCTGGGCTGCCA
GCGCAGGCCTCCGCGCAAACACAGCAGGCTGGAAGTGGCGCTCATCACCGGCACGTCTTCCCAG3′

Bisulfite-converted target DNA with primer and Flap oligo. sites: (SEQ ID NO:385)

5′GGTTATATAGGTTTATTTTGGTTTTTTGAGTTTTCGGCGGATTTAGGGTATTTAAGGAGCGGTTTTGGGTTGTTA
GCGTAGGTTTTCGCGTAAATATAGTAGGTTGGAAGTGGCGTTTATTATCGGTACGTTTTTTTAG3′

PCR and Flap Assay oligonucleotides:

B3GALT6 Forward Primer: 5′GGTTTATTTTGGTTTTTTGAGTTTTCGG3′ (SEQ ID NO:386)
B3GALT6 Reverse Primer: 5′TCCAACCTACTATATTTACGCGAA3′ (SEQ ID NO:387)
B3GALT6 Flap oligo.: 5′CCACGGACGGCGGATTTAGGG/3C6/ (SEQ ID NO:388)
B3GALT6 Flap oligonucleotide v2.: 5′ACGGACGCGGAGGCGGATTTAGGGTATTTAAGGAG/3C6/ (SEQ ID NO:436)

FIG. 1 (cont'd)

BARX1 Target DNA: (SEQ ID NO:21)

5′GGCCCGGGGCCGCCTGGGCCCCTAGGGGCTGGACGTCAACCTGTTAGATAGAGGGCGTGGGACCCCCCGCAGGCG
GCTGCTCGGACGACCGCATCCGGAG3′

Bisulfite-converted Target DNA: (SEQ ID NO:22)

5′GGTTCGGGGTCGTTTGGGTTTTTAGGGGTTGGACGTTAATTTGTTAGATAGAGGGCGTGGGATTTTTCGTAGGCG
GTTGTTCGGACGATCGTATTCGGAG3′

PCR and Flap Assay Oligonucleotides:

BARX1 Forward Primer: 5′CGTTAATTTGTTAGATAGAGGGCG3′ (SEQ ID NO:23)
BARX1 Reverse Primer: 5′ACGATCGTCCGAACAACC3′ (SEQ ID NO:24)
BARX1 Flap oligo.: 5′CCACGGACGCGCCTACGAAAA/3C6/ (SEQ ID NO:25)

BARX1 Forward Primer: 5′CGTTAATTTGTTAGATAGAGGGCG3′ SEQ ID NO:23)
BARX1 Reverse Primer Universal: 5′ TCCGAACAACCGCCTAC3′(SEQ ID NO:26)
BARX1 Flap oligo. Universal: 5′ CCACGGACGCGAAAAATCCCA/3C6/ (SEQ ID NO:27)
BARX1_Flap oligo._v6: 5′ AGGCCACGGACGCGAAAAATCCCACGC/3C6/ (SEQ ID NO: 405)

FIG. 1 (cont'd)

BCAT1 Target DNA: (SEQ ID NO:28)

5'GCTTCCAGCCGCGCGCTCCGTGCCACTGCCGCTCTCTGCAGCCCCGCGTCCCCGCAGCCTCCCCATGGCCAGCCC
GCTTCGCTCCGCTGCGGCCCTTGCCCGCCAGGTACCTCGAACCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:29)

5'GTTTTTAGTCGCGCGTTTCGTGTTATTGTCGTTTTTTGTAGTTTCGCGTTTTCGTAGTTTTTTTATGGTTAGTTC
GTTTCGTTTCGTTGCGGTTTTTGTTCGTTAGGTATTTCGAATTT3'

PCR and Flap Assay Oligonucleotides:

BCAT1 Forward Primer:	5'GTGTTATTGTCGTTTTTTGTAGTTTCG3' (SEQ ID NO:30)
BCAT1 Reverse Primer:	5'CGCAACGAAACGAAACGA3' (SEQ ID NO:31)
BCAT1 Flap oligo.:	5'CGCCGAGGGCGTTTTCGTAG/3C6/ (SEQ ID NO:32)

FIG. 1 (cont'd)

BCL2L11 Target DNA: (SEQ ID NO:33)

5'GCCCGCCGCACGCCGCAATGCTCCGCGCTCCCCGCGGGGTCGGGCGACTCAGACAGGGACCGGAAAAGAACCACG
CAGAAGAAAGCCCTATTTCTTGTCGTCTGTTCCTGTGCAGCCTTGCAGCCTCGCCGCCCCCGCGT3'

Bisulfite-converted Target DNA: (SEQ ID NO:34)

5'GTTCGTCGTACGTCGTAATGTTTCGCGTTTTTCGCGGGGTCGGGCGATTTAGATAGGGATCGGAAAAGAATTACG
TAGAAGAAAGTTTTATTTTTTGTCGTTTGTTTTTGTGTAGTTTTGTAGTTTCGTCGTTTTCGCGT3'

PCR and Flap Assay Oligonucleotides:

BCL2L11 Forward Primer: 5'CGTAATGTTTCGCGTTTTTCG3' (SEQ ID NO:35)
BCL2L11 Reverse Primer: 5'ACTTTCTTCTACGTAATTCTTTTCCGA3' (SEQ ID NO:36)
BCL2L11 Flap oligo.: 5'CGCCGAGGGCGGGGTCGGGC/3C6/ (SEQ ID NO:37)

FIG. 1 (cont'd)

BHLHE23 Target DNA (SEQ ID NO:38)

5′GCCGGGGAGTCGAGAAGCAAGTACTAGCGCTCCAGGACCGCGCGCGCCGCCCCGCGCCGCCCCGCGCCGCCCCTC
GGTCCAGAGC3′

Bisulfite-converted Target DNA: (SEQ ID NO:39)

5′GTCGGGGAGTCGAGAAGTAAGTATTAGCGTTTTAGGATCGCGCGCGTCGTTTCGCGTCGTTTCGCGTCGTTTTTC
GGTTTAGAGT3′

PCR and Flap Assay Oligonucleotides:

BHLHE23_Forward Primer:	5′AGTATTAGCGTTTTAGGATCGCG3′ (SEQ ID NO:40)
BHLHE23_Reverse Primer:	5′ACTCTAAACCGAAAAACGACG3′ (SEQ ID NO:41)
BHLHE23_Flap oligo.:	5′CCACGGACGGCGAAACGACGC/3C6/ (SEQ ID NO:42)

FIG. 1 (cont'd)

BIN2_HM Target DNA: (SEQ ID NO:43)

5'GCCGGGAGCCCGCACTTCCTCCTCGGGGGCCTCAGAAAACCACAGGGCGCGGGGCCAGGGCGGCGGCCCC
CAGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:44)

5'GTCGGGAGTTCGTATTTTTTTTTCGGGGGTTTTAGAAAATTATAGGGCGCGGGGTTAGGGCGGCGGTTTT
TAGG3'

PCR and Flap Assay Oligonucleotides:

BIN2_HM Forward Primer:	5'TCGGGAGTTCGTATTTTTTTTTCGG3' (SEQ ID NO:45)
BIN2_HM Reverse Primer:	5'AAAACCGCCGCCCTAAC3' (SEQ ID NO:46)
BIN2_HM Flap oligo.:	5'CGCCGAGGCCCCGCGCCCTA/3C6/ (SEQ ID NO:47)

FIG. 1 (cont'd)

BIN2_Z Target DNA: (SEQ ID NO:48)

5′CGGGGCCTACCCTCAGGCAGCGCTCGCTCGAGGCCAGCTTCCGAGCTCCAACCCCTGCCCGAAACCTCGGCCTCA
CTG3′

Bisulfite-converted Target DNA: (SEQ ID NO:49)

5′CGGGGTTTATTTTTAGGTAGCGTTCGTTCGAGGTTAGTTTTCGAGTTTTAATTTTTGTTCGAAATTTCGGTTTTA
TTG3′

PCR and Flap Assay Oligonucleotides:

BIN2_Z Forward Primer: 5′GGGTTTATTTTTAGGTAGCGTTCG3′ (SEQ ID NO:50)
BIN2_Z Reverse Primer: 5′CGAAATTTCGAACAAAAATTAAAACTCGA3′ (SEQ ID NO:51)
BIN2_Z Flap oligo.: 5′CCACGGACGGTTCGAGGTTAG/3C6/ (SEQ ID NO:52)

FIG. 1 (cont'd)

CAPN2 Target DNA (SEQ ID NO:53)

5'TGTCCTGACACGATGGCCACAGGCACAGTTTGTGGTGATGCCCAGGGGCCCGCGCGGCCCCACGGTGGTCCAGTT
TACACTCGGGCCCCGCACTCCTGAAGTTCCGCGCGGGAGGAGAAGGGCGTCCCTTTCGCAGCTCGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:54)

5'TGTTTTGATACGATGGTTATAGGTATAGTTTGTGGTGATGTTTAGGGGTTCGCGCGGTTTTACGGTGGTTTAGTT
TATATTCGGGTTTCGTATTTTTGAAGTTTCGCGCGGGAGGAGAAGGGCGTTTTTTTCGTAGTTCGG3'

PCR and Flap Assay Oligonucleotides:

CAPN2 Forward Primer:	5'TGATGTTTAGGGGTTCGCG3' (SEQ ID NO:55)
CAPN2 Reverse Primer:	5'CGAAACTTCAAAAATACGAAACCCGA3' (SEQ ID NO:56)
CAPN2 Flap oligo.:	5'CGCCGAGGGCGGTTTTACGG/3C6/ (SEQ ID NO:57)

FIG. 1 (cont'd)

chr5_132 Target DNA: (SEQ ID NO:58)

5'CCGGAGCACTCGCCGCTGCGCGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAGGCCGGCGGGCTGGGCGGCTC
GGCAGCCTGCGCCGCGGCCTCCGCCTCGGCCGCCAGC3'

Bisulfite-converted Target DNA: (SEQ ID NO:59)

5'TCGGAGTATTCGTCGTTGCGCGTTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAGGTCGGCGGGTTGGGCGGTTC
GGTAGTTTGCGTCGCGGTTTTCGTTTCGGTCGTTAGT3'

PCR and Flap Assay Oligonucleotides:

chr5_132 Forward Primer: 5'GTATTCGTCGTTGCGCG3' (SEQ ID NO:60)
chr5_132 Reverse Primer: 5'CCTCGAAAACCGCCTACC3' (SEQ ID NO:61)
chr5_132 Flap oligo.: 5'CCACGGACGCGCCAACGACTT/3C6/ (SEQ ID NO:62)

FIG. 1 (cont'd)

chr7_636 Target DNA: (SEQ ID NO:63)

5'CGCCGTGAGTGTTATAGTTCTTAAAGGCGGCGTGTCCGGAGTTTCTTCCTTCTGGTGGGGTTCGTGGTCTCGCCG
GCTCAGGAGTGAAGCTGCAGATCTTCGCGGTGAGTGTTACAGCTCCTAAGGCGGCGCAT3'

Bisulfite-converted Target DNA: (SEQ ID NO:64)

5'CGTCGTGAGTGTTATAGTTTTTAAAGGCGGCGTGTTCGGAGTTTTTTTTTTTTGGTGGGGTTCGTGGTTTCGTCG
GTTTAGGAGTGAAGTTGTAGATTTTCGCGGTGAGTGTTATAGTTTTTAAGGCGGCGTAT3'

PCR and Flap Assay Oligonucleotides:

chr7_636_HM Forward Primer: 5'TAAAGGCGGCGTGTTCG3' (SEQ ID NO:65)
chr7_636_HM Reverse Primer: 5'CAACTTCACTCCTAAACCGAC3' (SEQ ID NO:66)
chr7_636_HM Flap oligo.: 5'CCACGGACGCGAAACCACGAA/3C6/ (SEQ ID NO:67)

FIG. 1 (cont'd)

CYP26C1 Target DNA: (SEQ ID NO:68)

5'AACTGGCCTTCTGGCTACTCCGGAATCGCCAAGCAGATGAGGCCAGACCGCCGCCAGCGCTGATCACGCGCGCTC
CCACAGGTCCTGGCGCGCGTGTTCAGCCGCGC3'

Bisulfite-converted Target DNA: (SEQ ID NO:69)

5'AATTGGTTTTTTGGTTATTTCGGAATCGTTAAGTAGATGAGGTTAGATCGTCGTTAGCGTTGATTACGCGCGTTT
TTATAGGTTTTGGCGCGCGTGTTTAGTCGCGT3'

PCR and Flap Assay Oligonucleotides:

CYP26C1 Forward Primer: 5'TGGTTTTTTGGTTATTTCGGAATCGT3' (SEQ ID NO:70)
CYP26C1 Reverse Primer: 5'GCGCGTAATCAACGCTAAC3' (SEQ ID NO:71)
CYP26C1 Flap oligo.: 5'CGCCGAGGCGACGATCTAAC/3C6/ (SEQ ID NO:72)

FIG. 1 (cont'd)

DIDO1 Target DNA: (SEQ ID NO:73)

5′GGAGCGGGCAGAGGAGGAGCCCAGCGCCGAGGCCCAGGCGCGCCCCGCCCTCGCCCCTCCCCGTGCCCCTCCCCC
GCTGCTCCCC3′

Bisulfite-converted Target DNA: (SEQ ID NO:74)

5′GGAGCGGGTAGAGGAGGAGTTTAGCGTCGAGGTTTAGGCGCGTTTCGTTTTCGTTTTTTTTCGTGTTTTTTTTTC
GTTGTTTTTT3′

PCR and Flap Assay Oligonucleotides:

DIDO1 Forward Primer:	5′GAGGAGGAGTTTAGCGTCG3′ (SEQ ID NO:75)
DIDO1 Reverse Primer:	5′CACGAAAAAAAACGAAAACGAAAC3′ (SEQ ID NO:76)
DIDO1 Flap oligo.:	5′CGCCGAGGCGCGCCTAAACC/3C6/ (SEQ ID NO:77)

FIG. 1 (cont'd)

DLX4 Target DNA: (SEQ ID NO:78)

5'GCGGTCTATCACGGGCACCCCTAACACTTGGTGAGTGCGCAGTGCTCTCGGCAGTCTCTGGGCTCCATACGATGC
CTACCGCACGCCCTAGCAGAGGAGGTCTCTGT3'

Bisulfite-converted Target DNA: (SEQ ID NO:79)

5'GCGGTTTATTACGGGTATTTTTAATATTTGGTGAGTGCGTAGTGTTTTCGGTAGTTTTTGGGTTTTATACGATGT
TTATCGTACGTTTTAGTAGAGGAGGTTTTTGT3'

PCR and Flap Assay Oligonucleotides:

DLX4 Forward Primer: 5'TGAGTGCGTAGTGTTTTCGG3' (SEQ ID NO:80)
DLX4 Reverse Primer: 5'CTCCTCTACTAAAACGTACGATAAACA3' (SEQ ID NO:81)
DLX4 Flap oligo.: 5'CGCCGAGGATCGTATAAAAC/3C6/ (SEQ ID NO:82)

DLX4 Forward Primer Universal: 5'ATATTTGGTGAGTGCGTAGTG3' (SEQ ID NO:83)
DLX4 Reverse Primer Universal: 5'ACGTACGATAAACATCGTATAAAACC3'(SEQ ID NO:84)
DLX4 Flap oligo. Universal: 5'CGCCGAGGGTTTTCGGTAGT/3C6/(SEQ ID NO:85)

FIG. 1 (cont'd)

DMRTA2 Target DNA: (SEQ ID NO:86)

5'TACTCCACTGCCGGCTTGGTGCCCACGCTCGGCTTCCGCCCACCCATGGACTACGCCTTTAGCGATCTCATGCGT
GACCGCTCGGCCGCCGCTGCTGCGGCGGTGCACAAGGAGCCGACCT3'

Bisulfite-converted Target DNA: (SEQ ID NO:87)

5'TATTTTATTGTCGGTTTGGTGTTTACGTTCGGTTTTCGTTTATTTATGGATTACGTTTTTAGCGATTTTATGCGT
GATCGTTCGGTCGTCGTTGTTGCGGCGGTGTATAAGGAGTCGATTT3'

PCR and Flap Assay Oligonucleotides:

DMRTA2 Forward Primer: 5'TGGTGTTTACGTTCGGTTTTCGT3' (SEQ ID NO:88)
DMRTA2 Reverse Primer: 5'CCGCAACAACGACGACC3' (SEQ ID NO:89)
DMRTA2 Flap oligo.: 5'CGCCGAGGCGAACGATCACG/3C6/ (SEQ ID NO:90)

FIG. 1 (cont'd)

DNMT3A Target DNA: (SEQ ID NO:91)

5'AGGCCGGTCACGAACAAAGCGCTGGCGAGTGCGCGCCCGCCCACGCGCACAGGTGCCCGCGACAAGACGCCCCGT
CCCCGCCCACGCGGCCCCCGCGGGCTGAGCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:92)

5'AGGTCGGTTACGAATAAAGCGTTGGCGAGTGCGCGTTCGTTTACGCGTATAGGTGTTCGCGATAAGACGTTTCGT
TTTCGTTTACGCGGTTTTCGCGGGTTGAGTT3'

PCR and Flap Assay Oligonucleotides:

DNMT3A Forward Primer: 5'GTTACGAATAAAGCGTTGGCG3' (SEQ ID NO:93)
DNMT3A Reverse Primer: 5'AACGAAACGTCTTATCGCGA3' (SEQ ID NO:94)
DNMT3A Flap oligo.: 5'CCACGGACGGAGTGCGCGTTC/3C6/ (SEQ ID NO:95)

FIG. 1 (cont'd)

DOCK2 Target DNA: (SEQ ID NO:96)

5'GCCGGCCCCGCAGCATCCTCCTGCTCGCGGCTCTCCCGCCACCTGTCCCGCTCCCTGCCGCGCCCTGGGGCCCGC
ACCTACCCAC3'

Bisulfite-converted Target DNA: (SEQ ID NO:97)

5'GTCGGTTTCGTAGTATTTTTTTGTTCGCGGTTTTTTCGTTATTTGTTTCGTTTTTTGTCGCGTTTTGGGGTTCGT
ATTTATTTAT3'

PCR and Flap Assay Oligonucleotides:

DOCK2 Forward Primer:	5'CGGTTTCGTAGTATTTTTTTGTTCG3' (SEQ ID NO:98)
DOCK2 Reverse Primer:	5'GAACCCCAAAACGCGAC3' (SEQ ID NO:99)
DOCK2 Flap oligo.:	5'CGCCGAGGGCGGTTTTTTCG/3C6/ (SEQ ID NO:100)

FIG. 1 (cont'd)

DTX1 Target DNA: (SEQ ID NO:101)

5′CGCCTCCTGGGCTCCCCCCGGAGTGGGAGGGAGCCGCGGTCCCGCCTCCGCGCCCGTTCCCTCCCAGGCCCCTCG
GCCGCCGCGCCGAGCTTTCCGCGCGTGGACAGACTGCCCGGCCGACGGACGGACGCAGG3′

Bisulfite-converted Target DNA: (SEQ ID NO:102)

5′CGTTTTTTGGGTTTTTTTCGGAGTGGGAGGGAGTCGCGGTTTCGTTTTCGCGTTCGTTTTTTTTTAGGTTTTTCG
GTCGTCGCGTCGAGTTTTTCGCGCGTGGATAGATTGTTCGGTCGACGGACGGACGTAGG3′

PCR and Flap Assay Oligonucleotides:

DTX1 Forward Primer_49: 5′GAGTCGCGGTTTCGTTTTC3′ (SEQ ID NO:103)
DTX1 Reverse Primer_Ver2: 5′GACGCGACGACCGAAAAAC3′ (SEQ ID NO:104)
DTX1 Flap oligo. S_49: 5′CGCCGAGGCGCGTTCGTTTT/3C6/ (SEQ ID NO:105)

FIG. 1 (cont'd)

EMX1 Target DNA: (SEQ ID NO:106)

5'TCCGGCGCCGCGTTTTCTAGAGAACCGGGTCTCAGCGATGCTCATTTCAGCCCCGTCTTAATGCAACAAACGAAA
CCCCACACGAACGAAAAGGAACATGTCTGCGCT3'

Bisulfite-converted Target DNA: (SEQ ID NO:107)

5'TCGGCGTCGCGTTTTTTAGAGAATCGGGTTTTAGCGATGTTTATTTTAGTTTCGTTTTAATGTAATAAACGAAAT
TTTATACGAACGAAAAGGAATATGTTTGCGTT3'

PCR and Flap Assay Oligonucleotides:

EMX1 Forward Primer: 5'GGCGTCGCGTTTTTTAGAGAA3' (SEQ ID NO:108)
EMX1 Reverse Primer: 5'TTCCTTTTCGTTCGTATAAAATTTCGTT3' (SEQ ID NO:109)
EMX1 Flap oligo.: 5'CCACGGACGATCGGGTTTTAG/3C6/ (SEQ ID NO:110)

FIG. 1 (cont'd)

FAM59B Target DNA: (SEQ ID NO:111)

5'GGGCCTGCTGGCCGGGGACCCGCGCGTCGAGCGCCTGGTGCGCGACAGCGCCTCCTACTGCCGCGAGCGCTTCGA
CCCCGACGAGTACTCCACGGCCGTGCGCGAGGCGCCAGCGGAGCTCGCCGAAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:112)

5'GGGTTTGTTGGTCGGGGATTCGCGCGTCGAGCGTTTGGTGCGCGATAGCGTTTTTTATTGTCGCGAGCGTTTCGA
TTTCGACGAGTATTTTACGGTCGTGCGCGAGGCGTTAGCGGAGTTCGTCGAAG3'

PCR and Flap Assay Oligonucleotides:

FAM59B Forward Primer: 5'CGATAGCGTTTTTTATTGTCGCG3' (SEQ ID NO:113)
FAM59B Reverse Primer: 5'GCACGACCGTAAAATACTCGTC3' (SEQ ID NO:114)
FAM59B Flap oligo.: 5'CCACGGACGCGAAATCGAAAC/3C6/ (SEQ ID NO:115)

FAM59B_Forward Primer v3: 5'GTCGAGCGTTTGGTGCG (SEQ ID NO:406)
FAM59B_Reverse Primer v3: 5'CTCGTCGAAATCGAAACGC (SEQ ID NO:407)
FAM59B_Flap oligo v3: 5'CGCGCCGAGGGCGATAGCGTTTTTTATTGTCG/3C6/(SEQ ID NO:408)

FIG. 1 (cont'd)

FERMT3 Target DNA: (SEQ ID NO:116)

5'TAGCAGCAGCCGCAGCCATGGCGGGGATGAAGACAGCCTCCGGGGACTACATCGACTCGTCATGGGAGCTGCGGG
TGTTTGTGGGAGAGGAGGACCCAGAGGCCGAGTCGGTCACCCTGCGGGTCACTGGGGAGTCGCAC3'

Bisulfite-converted Target DNA: (SEQ ID NO:117)

5'TAGTAGTAGTCGTAGTTATGGCGGGGATGAAGATAGTTTTCGGGGATTATATCGATTCGTTATGGGAGTTGCGGG
TGTTTGTGGGAGAGGAGGATTTAGAGGTCGAGTCGGTTATTTTGCGGGTTATTGGGGAGTCGTAT3'

PCR and Flap Assay Oligonucleotides:

FERMT3 Forward Primer:	5'GTTTTCGGGGATTATATCGATTCG3' (SEQ ID NO:118)
FERMT3 Reverse Primer:	5'CCCAATAACCCGCAAAATAACC3' (SEQ ID NO:119)
FERMT3 Flap oligo.:	5'CGCCGAGGCGACTCGACCTC/3C6/ (SEQ ID NO:120)

FIG. 1 (cont'd)

FGF14 Target DNA: (SEQ ID NO:121)

5'GTCCCAGAGACGCCCTAGGGTCAGAGGTCATCTCCGTGGCAACGGAAACTTCCCGCGCTACGGCGGCTCCAACGG
GCCGCTTCCGCCGCATTGCGTAGCGAAGC3'

Bisulfite-converted Target DNA: (SEQ ID NO:122)

5'GTTTTAGAGACGTTTTAGGGTTAGAGGTTATTTTCGTGGTAACGGAAATTTTTCGCGTTACGGCGGTTTTAACGG
GTCGTTTTCGTCGTATTGCGTAGCGAAGT3'

PCR and Flap Assay Oligonucleotides:

FGF14 Forward Primer: 5'TTTCGTGGTAACGGAAATTTTTCG3' (SEQ ID NO:123)
FGF14 Reverse Primer: 5'CGACGAAAACGACCCGT3' (SEQ ID NO:124)
FGF14 Flap oligo.: 5'CGCCGAGGGCGTTACGGCGG/3C6/ (SEQ ID NO:125)

FIG. 1 (cont'd)

FLJ34208 Target DNA: (SEQ ID NO:126)

5'GCGCCCCGGCCGCAGGCGGAGGACAGGGAGGAGCGCACACGAGAAAGCTCCCACGCGCCCGCGCCTCGCCTCCGA
CGGGAAGGCGCCTCTTCCGACCGTCCTGGATG3'

Bisulfite-converted Target DNA: (SEQ ID NO:127)

5'GCGTTTCGGTCGTAGGCGGAGGATAGGGAGGAGCGTATACGAGAAAGTTTTTACGCGTTCGCGTTTCGTTTTCGA
CGGGAAGGCGTTTTTTTCGATCGTTTTGGATG3'

PCR and Flap Assay Oligonucleotides:

FLJ34208 Forward Primer: 5'GAGCGTATACGAGAAAGTTTTTACG (SEQ ID NO:128)
FLJ34208 Reverse Primer: 5'AACGCCTTCCCGTCGAA (SEQ ID NO:129)
FLJ34208 Flap oligo.: 5'CCACGGACGGCGTTCGCGTTT/3C6/ (SEQ ID NO:130)

FIG. 1 (cont'd)

FLJ45983 Target DNA: (SEQ ID NO:131)

5'CGAGAGGGCGCGAGCACAGCCGAGGCCATGGAGGTGACGGCGGACCAGCCGCGCTGGGTGAGCCACCACCACCCC
GCCGTGCTCAACGGGCAGCACCCGGACACGCAC3'

Bisulfite-converted Target DNA: (SEQ ID NO:132)

5'CGAGAGGGCGCGAGTATAGTCGAGGTTATGGAGGTGACGGCGGATTAGTCGCGTTGGGTGAGTTATTATTATTTC
GTCGTGTTTAACGGGTAGTATTCGGATACGTAT3'

PCR and Flap Assay Oligonucleotides:

FLJ45983 Forward Primer: 5'GGGCGCGAGTATAGTCG3' (SEQ ID NO:133)
FLJ45983 Reverse Primer: 5'CAACGCGACTAATCCGC3' (SEQ ID NO:134)
FLJ45983 Flap oligo.: 5'CGCCGAGGCCGTCACCTCCA/3C6/ (SEQ ID NO:135)

FLJ45983 Forward Primer v4 5'CGAGGTTATGGAGGTGACG (SEQ ID NO:409)
FLJ45983 Reverse Primer v4 5'CGAATACTACCCGTTAAACACG (SEQ ID NO:410)
FLJ45983 Flap oligo. v4:_ 5'AGGCCACGGACGGGCGGATTAGTCGCG/3C6/ (SEQ ID NO:411)

FIG. 1 (cont'd)

GRIN2D Target DNA: (SEQ ID NO:136)

5'CGCCCCCTCACCTCCCCGATCATGCCGTTCCAGACGCCATCGATCTTCTTTCCGTGCTTGCCATTGGTGACCAGG
TAGAGGTCGTAGCTGAAGCCGATGGTATGCGCCAGCCGCTTCAGAATGTCGATGCAGAAACCCTTG3'

Bisulfite-converted Target DNA: (SEQ ID NO:137)

5'CGTTTTTTTATTTTTTCGATTATGTCGTTTTAGACGTTATCGATTTTTTTTTCGTGTTTGTTATTGGTGATTAGG
TAGAGGTCGTAGTTGAAGTCGATGGTATGCGTTAGTCGTTTTAGAATGTCGATGTAGAAATTTTTG3'

PCR and Flap Assay Oligonucleotides:

GRIN2D Forward Primer: 5'TCGATTATGTCGTTTTAGACGTTATCG3' (SEQ ID NO:138)
GRIN2D Reverse Primer: 5'TCTACATCGACATTCTAAAACGACTAAC3'(SEQ ID NO:139)
GRIN2D Flap oligo.: 5'CCACGGACGCGCATACCATCG/3C6/(SEQ ID NO:140)

FIG. 1 (cont'd)

HIST1H2BE Target DNA: (SEQ ID NO:141)

5'CGGCGAGGCTTCCCGCCTGGCGCATTACAACAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCG
CCTGCTGCTTCCCGGGGA3'

Bisulfite-converted Target DNA: (SEQ ID NO:142)

5'CGGCGAGGTTTTTCGTTTGGCGTATTATAATAAGCGTTCGATTATTATTTTTAGGGAGATTTAGACGGTCGTGCG
TTTGTTGTTTTTCGGGGA3'

PCR and Flap Assay Oligonucleotides:

HIST1H2BE Forward Primer: 5'TGGCGTATTATAATAAGCGTTCG3' (SEQ ID NO:143)
HIST1H2BE Reverse Primer: 5'AACAACAAACGCACGACC3' (SEQ ID NO:144)
HIST1H2BE Flap oligo.: 5'CCACGGACGCGTCTAAATCTC/3C6/ (SEQ ID NO:145)

FIG. 1 (cont'd)

HOPX Target DNA (SEQ ID NO:412)

5'GGCGGCGCCGCGACCGCCTTCCTTCGCTGCGTCCCGCCCGCTCCACGCCTCGCTCACCGCCGCCGCTTCTCCCTG
CCCCGCAGCGCGCAGGGACCATGTCGGCGGAGACCGCGAGCGGCCCCACAGAGGACCAGGTGGAAATCCTGGAGTAC
AACTTCAACAAGGTCGACAAGCACCCGGATTCCACCACGCTGTGCCTCATCGCGGCCGAGGCAGGCCTTTCCGAGGA
GGAGACCCAGGTGCGTCCCCACACGCGCCCAGCGCGCCCCGACCCCTGCCTGGGCTGAGCCTTCTCGCGGCTGGGCG
GTCCTGTTCGTCCGCGCCTCCCGC3'

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Bisulfite-converted Target DNA: (SEQ ID NO:413)

5'GGCGGCGTCGCGATCGTTTTTTTTCGTTGCGTTTCGTTCGTTTTACGTTTCGTTTATCGTCGTCGTTTTTTTTTG
TTTCGTAGCGCGTAGGGATTATGTCGGCGGAGATCGCGAGCGGTTTTATAGAGGATTAGGTGGAAATTTTGGAGTAT
AATTTTAATAAGGTCGATAAGTATTCGGATTTTATTACGTTGTGTTTTATCGCGGTCGAGGTAGGTTTTTTCGAGGA
GGAGATTTAGGTGCGTTTTTATACGCGTTTAGCGCGTTTCGATTTTTGTTTGGGTTGAGTTTTTTCGCGGTTGGGCG
GTTTTGTTCGTTCGCGTTTTTCGT3'

FIG. 1 (cont'd)

HOPX assays continued

PCR and Flap Assay Oligonucleotides:

HOPX_2236 Forward Primer 5'GCGTCGCGATCGTTTTTTTTCG (SEQ ID NO:414)
HOPX_2236 Reverse Primer 5'AACGACGACGATAAACGAAACGTA (SEQ ID NO:415)
HOPX_2236 Flap oligo. 5'AGGCCACGGACG GTTGCGTTTCGTTCGTTT/3C6/ (SEQ ID NO:416)

HOPX_2149 Forward Primer 5'GTAGCGCGTAGGGATTATGTCG (SEQ ID NO:417)
HOPX_2149 Reverse Primer 5'TTTCCACCTAATCCTCTATAAAACCGC (SEQ ID NO:418)
HOPX_2149 Flap oligo. 5'AGGCCACGGACG CTCGCGATCTCCGC/3C6/ (SEQ ID NO:419)

HOPX Forward Primer 5'ACGTTGTGTTTTATCGCGG (SEQ ID NO:420)
HOPX Reverse Primer 5'CTAAACGCGTATAAAAACGCAC (SEQ ID NO:421)
HOPX Flap oligo. 5'AGGCCACGGACG GTCGAGGTAGGTTTTTTCGA (SEQ ID NO:422)

FIG. 1 (cont'd)

HOXA9 Target DNA: (SEQ ID NO:146)

5'GGGCGGGCCAGGCGCTGGGCACGGTGATGGCCACCACTGGGGCCCTGGGCAACTACTACGTGGACTCGTTCCTGC
TGGGCGCCGACGCCGCGGATGAGCTG3'

Bisulfite-converted Target DNA: (SEQ ID NO:147)

5'GGGCGGGTTAGGCGTTGGGTACGGTGATGGTTATTATTGGGGTTTTGGGTAATTATTACGTGGATTCGTTTTTGT
TGGGCGTCGACGTCGCGGATGAGTTG3'

PCR and Flap Assay Oligonucleotides:

HOXA9 Forward Primer: 5'TTGGGTAATTATTACGTGGATTCG3' (SEQ ID NO:148)
HOXA9 Reverse Primer: 5'ACTCATCCGCGACGTC3' (SEQ ID NO:149)
HOXA9 Flap oligo.: 5'CCACGGACGCGACGCCCAACA/3C6/ (SEQ ID NO:150)

HOXA9 Forward Primer: 5'TTGGGTAATTATTACGTGGATTCG (SEQ ID NO:148)
HOXA9 Reverse Primer v2: 5'CAACTCATCCGCGACG (SEQ ID NO:423)
HOXA9 Flap oligo.: v2 5'AGGCCACGGACGGTCGACGCCCAACAA/3C6/(SEQ ID NO:424)

FIG. 1 (cont'd)

HOXB2 Target DNA: (SEQ ID NO:151)

5'GGGCCATTGCCAGAAGACGTCTTCTCGGGGCGCCAGGATTCACCTTTCCTTCCCGACCTCAACTTCTTCGCGGCC
GACTCCTGTCTCCAGCTATC3'

Bisulfite-converted Target DNA: (SEQ ID NO:152)

5'GGGTTATTGTTAGAAGACGTTTTTTCGGGGCGTTAGGATTTATTTTTTTTTTTCGATTTTAATTTTTTCGCGGTC
GATTTTTGTTTTTAGTTATT3'

PCR and Flap Assay Oligonucleotides:

HOXB2 Forward Primer: 5'GTTAGAAGACGTTTTTTCGGGG3' (SEQ ID NO:153)
HOXB2 Reverse Primer: 5'AAAACAAAAATCGACCGCGA3' (SEQ ID NO:154)
HOXB2 Flap oligo.: 5'CGCCGAGGGCGTTAGGATTT/3C6/ (SEQ ID NO:155)
HOXB2 Flap oligo. 2: 5'CGCGCCGAGGGCGTTAGGATTTATTTTTTTTTTTCGA/3C6/
(SEQ ID NO:425)

FIG. 1 (cont'd)

IFFO1 Target DNA (SEQ ID NO:426)

5′ CGGGACAGAGCCGACCAATCAGGCGGCTCGGCAGCGGGGCAGAGGTCAGGGGGCGGGCCGAGGGGAAGCCAA3′

Bisulfite-converted Target DNA: (SEQ ID NO:427)

5′ CGGGATAGAGTCGATTAATTAGGCGGTTCGGTAGCGGGGTAGAGGTTAGGGGGCGGGTCGAGGGGAAGTTAA3′

PCR and Flap Assay Oligonucleotides:

IFFO1 Forward Primer: 5′CGGGATAGAGTCGATTAATTAGGC (SEQ ID NO:428)
IFFO1 Reverse Primer: 5′TAACTTCCCCTCGACCCG (SEQ ID NO:429)
IFFO1 Flap oligo. 5′CGCGCCGAGGCGGTTCGGTAGCGG/3C6/ (SEQ ID NO:430)

FIG. 1 (cont'd)

KLHDC7B.chr22.50987185-50987290 Target DNA: (SEQ ID NO:156)

5'GGCCCCGGAAGCCCAGCTCCCGGGCCCTGGAGCCCGCCACGGCGGCAGCCCTGCGGCGGCGGCTGGACCTGGGCA
GTTGCCTGGACGTGCTGGCCTTTGCCCAGCA3'

Bisulfite-converted Target DNA: (SEQ ID NO:157)

5'GGTTTCGGAAGTTTAGTTTTCGGGTTTTGGAGTTCGTTACGGCGGTAGTTTTGCGGCGGCGGTTGGATTTGGGTA
GTTGTTTGGACGTGTTGGTTTTTGTTTAGTA3'

PCR and Flap Assay Oligonucleotides:

KLHDC7B Forward Primer: 5'AGTTTTCGGGTTTTGGAGTTCGTTA3' (SEQ ID NO:158)
KLHDC7B Reverse Primer: 5'CCAAATCCAACCGCCGC3' (SEQ ID NO:159)
KLHDC7B Flap oligo.: 5'CGCCGAGGACGGCGGTAGTT/3C6/ (SEQ ID NO:160)

FIG. 1 (cont'd)

LOC100129726 Target DNA: (SEQ ID NO:161)

5'GGCGGCGCCGGCGGCTGCGCGGGGGGCGCCAGGCCCTGCTGCTGCTGCTGCTGCTGACTGCGGTAGTAGGCGGCG
GCGGCCACGGCGGCAAAGTTGTGGGTCTGGA

Bisulfite-converted Target DNA: (SEQ ID NO:162)

5'GGCGGCGTCGGCGGTTGCGCGGGGGGCGTTAGGTTTTGTTGTTGTTGTTGTTGTTGATTGCGGTAGTAGGCGGCG
GCGGTTACGGCGGTAAAGTTGTGGGTTTGGA

PCR and Flap Assay Oligonucleotides:

LOC100129726 Forward Primer: 5'TTGATTGCGGTAGTAGGCG3' (SEQ ID NO:163)
LOC100129726 Reverse Primer: 5'AACCCACAACTTTACCGCC3' (SEQ ID NO:164)
LOC100129726 Flap oligo.: 5'CGCCGAGGCGTAACCGCCGC/3C6/(SEQ ID NO:165)

FIG. 1 (cont'd)

MATK Target DNA: (SEQ ID NO:166)

5'GGTTTCCCCCCACCCCGGCCTCGGGGTCTCTCCACGTCTCCCCGCCGACGTGCTCACCTGCTCAGGGGGCGCCCC
CGAGCCGCGCCCCGCGCCCGCCCCCAGGAGGGCCTCCGCGAGCCGGCTGCACACCCCGAGGCGGTCCCGGCTGCACA
AC3'

Bisulfite-converted Target DNA: (SEQ ID NO:167)

5'GGTTTTTTTTTATTTCGGTTTCGGGGTTTTTTTACGTTTTTTCGTCGACGTGTTTATTTGTTTAGGGGGCGTTTT
CGAGTCGCGTTTCGCGTTCGTTTTTAGGAGGGTTTTCGCGAGTCGGTTGTATATTTCGAGGCGGTTTCGGTTGTATA
AT3'

PCR and Flap Assay Oligonucleotides:

MATK Forward Primer: 5'GTTTCGGGGTTTTTTTACGTTTTTTCG3' (SEQ ID NO:168)
MATK Reverse Primer: 5'AAACGCGACTCGAAAACGC3' (SEQ ID NO:169)
MATK Flap oligo.: 5'CGCCGAGGGTCGACGTGTTT/3C6/ (SEQ ID NO:170)

FIG. 1 (cont'd)

MAX.chr10.22541891-22541946 Target DNA: (SEQ ID NO:171)

5'CTCCGGTTTTCGCGGTTCTCAGCGATATTAGGCGCGGCCAGTGTCTGAAAGCTCCTCGGGGTTACGTCCTGGGGC
GACTGGAGGCGGCTCACGAC3'

Bisulfite-converted Target DNA: (SEQ ID NO:172)

5'TTTCGGTTTTCGCGGTTTTTAGCGATATTAGGCGCGGTTAGTGTTTGAAAGTTTTTCGGGGTTACGTTTTGGGGC
GATTGGAGGCGGTTTACGAT3'

PCR and Flap Assay Oligonucleotides:

MAX_Chr10.225 Forward Primer: 5'CGGTTTTTAGCGATATTAGGCG3'(SEQ ID NO:173)
MAX_Chr10.225 Reverse Primer: 5'CCCAAAACGTAACCCCGA3'(SEQ ID NO:174)
MAX_Chr10.225 Flap oligo.: 5'CGCCGAGGGCGGTTAGTGTT/3C6/(SEQ ID NO:175)

FIG. 1 (cont'd)

MAX.chr10.22624430-22624544 Target DNA: (SEQ ID NO:176)

5′CGACGGCCGCGGAGGAGGAAGGCCAGGGGGAAATTTGCATTTCGTAAAACCGCGGTTAAGAAATGACGATGCCAC
GTAGACAAGCCAGTTGTGACGTTCAGCACAACGTGCTACTGAACTACCGAGATCCGCCACCAAATGGC3′

Bisulfite-converted Target DNA: (SEQ ID NO:177)

5′CGACGGTCGCGGAGGAGGAAGGTTAGGGGGAAATTTGTATTTCGTAAAATCGCGGTTAAGAAATGACGATGTTAC
GTAGATAAGTTAGTTGTGACGTTTAGTATAACGTGTTATTGAATTATCGAGATTCGTTATTAAATGGT3′

PCR and Flap Assay Oligonucleotides:

MAX_Chr10.226 Forward Primer: 5′GGGAAATTTGTATTTCGTAAAATCG3′ (SEQ ID NO:178)
MAX_Chr10.226 Reverse Primer: 5′ACAACTAACTTATCTACGTAACATCGT3′(SEQ ID NO:179)
MAX_Chr10.226 Flap oligo.: 5′CCACGGACGGCGGTTAAGAAA/3C6/ (SEQ ID NO:180)

FIG. 1 (cont'd)

MAX.chr12.52652268-52652362 Target DNA: (SEQ ID NO:181)

5'GGCTTGGGGTCCAGCCGCCCGCCCCTGCCGCCACCGCACCATGTCCTGCCTCTACTCCCGCCTCAGCGCCCCCTG
CGGGGTCCGCGCCTTCAGCTGCATCTCGGCCTGCGGGCCCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:182)

5'GGTTTGGGGTTTAGTCGTTCGTTTTTGTCGTTATCGTATTATGTTTTGTTTTTATTTTCGTTTTAGCGTTTTTTG
CGGGGTTCGCGTTTTTAGTTGTATTTCGGTTTGCGGGTTTT3'

PCR and Flap Assay Oligonucleotides:

MAX.chr12.52 Forward Primer: 5'TCGTTCGTTTTTGTCGTTATCG3' (SEQ ID NO:183)
MAX.chr12.52 Reverse Primer: 5'AACCGAAATACAACTAAAAACGC3'(SEQ ID NO:184)
MAX.chr12.52 Flap oligo.: 5'CCACGGACGCGAACCCCGCAA/3C6/(SEQ ID NO:185)

FIG. 1 (cont'd)

MAX.chr16.50875223-50875241 Target DNA: (SEQ ID NO:186)

5'GGAAGGCTGCAGCGAGAGATTTACATATTCATCCGAGCTTAAGGAAGCCGCGATAATGCAGGTACAGCCCGAAAC
CCACGCCCCCAGACCTTATCTGCGCGCCCCGCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:187)

5'GGAAGGTTGTAGCGAGAGATTTATATATTTATTCGAGTTTAAGGAAGTCGCGATAATGTAGGTATAGTTCGAAAT
TTACGTTTTTAGATTTTATTTGCGCGTTTCGTT3'

PCR and Flap Assay Oligonucleotides:

MAX.chr16.50 Forward Primer v2: 5'TTCGAGTTTAAGGAAGTCG3' (SEQ ID NO:188)
MAX.chr16.50 Reverse Primer v2: 5'TCTAAAAACGTAAATTTCGAACT3'(SEQ ID NO:189)
MAX.chr16.50 Flap oligo.: 5'CCACGGACGGCGATAATGTAG/3C6/(SEQ ID NO:190)

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FIG. 1 (cont'd)

MAX.chr19.16394489-16394575 Target DNA: (SEQ ID NO:191)

5'GGAGTTATTTTTAACCATCGCCTCCCAGAACATTACGGAGCTTCCTCTCTCCAACACGCAGGAAACCCTACTTGG
CTGTGCTTCCTGCTAACACGAGGCCCTGCGATTGCTGAGAACAACAGCCCCGAGACTGCGCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:192)

5'GGAGTTATTTTTAATTATCGTTTTTTAGAATATTACGGAGTTTTTTTTTTTTAATACGTAGGAAATTTTATTTGG
TTGTGTTTTTTGTTAATACGAGGTTTTGCGATTGTTGAGAATAATAGTTTCGAGATTGCGCG3'

PCR and Flap Assay Oligonucleotides:

MAX.chr19.16 For. Primer: 5'TTTAATTATCGTTTTTTAGAATATTACGGA3'(SEQ ID NO:193)
MAX.chr19.16 Reverse Primer: 5'ACTATTATTCTCAACAATCGCAAAAC3'(SEQ ID NO:194)
MAX.chr19.16 Flap oligo.: 5'CCACGGACGCCTCGTATTAAC/3C6/(SEQ ID NO:195)

FIG. 1 (cont'd)

MAX.chr19.37288426-37288480 Target DNA: (SEQ ID NO:196)

5'GGCGGGCGCTTGGCCAAACAGCCCAAGACTGCGGAATCACACTCGCCACTGTGTACCTGGACGCCATCTGCAGAC
CCAGCGCCTGCGGGGATTCCGGAAACGGGAGAGCGGGCTTCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:197)

5'GGCGGGCGTTTGGTTAAATAGTTTAAGATTGCGGAATTATATTCGTTATTGTGTATTTGGACGTTATTTGTAGAT
TTAGCGTTTGCGGGGATTTCGGAAACGGGAGAGCGGGTTTTT3'

PCR and Flap Assay Oligonucleotides:

MAX.chr19.37 For. Primer_v2: 5'AGTTTAAGATTGCGGAATTATATTCGT3'(SEQ ID NO:198)
MAX.chr19.37 Reverse Primer: 5'TTCCGAAATCCCCGCAA3' (SEQ ID NO:199)
MAX.chr19.37 Flap oligo.: 5'CGCCGAGGAACGCTAAATCT/3C6/ (SEQ ID NO:200)

FIG. 1 (cont'd)

MAX.chr8.124173236-124173370 Target DNA: (SEQ ID NO:201)

5'CGCAGGCTGAGGCCCTCGGGTCCCCAGCGGGTCCTCGCCATCAGTCACTCTCTACGGGCCAGGCCTGGGGGTCAC
GGCCTGCAGGAGCCTCCCTGCGCGGCCCCACTCCCTCATCTGCGACCCCGTGGGGAGGCGACCCTGACCACCCTCGT
TCCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:202)

5'CGTAGGTTGAGGTTTTCGGGTTTTTAGCGGGTTTTCGTTATTAGTTATTTTTTACGGGTTAGGTTTGGGGGTTAC
GGTTTGTAGGAGTTTTTTTGCGCGGTTTTATTTTTTTATTTGCGATTTCGTGGGGAGGCGATTTTGATTATTTTCGT
TTCG3'

PCR and Flap Assay Oligonucleotides:

MAX_Chr8.124 Forward Primer: 5'GGTTGAGGTTTTCGGGTTTTTAG3' (SEQ ID NO:203)
MAX_Chr8.124 Reverse Primer: 5'CCTCCCCACGAAATCGC3' (SEQ ID NO:204)
MAX_Chr8.124 Flap oligo.: 5'CGCCGAGGGCGGGTTTTCGT/3C6/ (SEQ ID NO:205)

MAX_Chr8.124 Forward Primer v2: 5'AGGAGTTTTTTTGCGCGG3' (SEQ ID NO:206)
MAX_Chr8.124 Reverse Primer v2: 5'ACGAAAATAATCAAAATCGCCTCC3' (SEQ ID NO:207)
MAX_Chr8.124 Flap oligo. v2: 5'CGCCGAGGCCCACGAAATCG/3C6/ (SEQ ID NO:208)

FIG. 1 (cont'd)

MAX.chr8.145105646-145105653 Target DNA (SEQ ID NO:209)

5'CGGGGGAGGGCGGCATCAGCCAGAGCCTCAGCCGACGGCGCTCCCCAGGTCCACTTCCCGCTCCGATACCCTCCC
CCTAAGCACGATACCCAGGGCCCAGGGCTGCTCTTGGCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:210)

5'CGGGGGAGGGCGGTATTAGTTAGAGTTTTAGTCGACGGCGTTTTTTAGGTTTATTTTTCGTTTCGATATTTTTTT
TTTAAGTACGATATTTAGGGTTTAGGGTTGTTTTTGGCG3'

PCR and Flap Assay Oligonucleotides:

MAX_Chr8.145 Forward Primer: 5'GCGGTATTAGTTAGAGTTTTAGTCG3' (SEQ ID NO:211)
MAX_Chr8.145 Reverse Primer: 5'ACAACCCTAAACCCTAAATATCGT3' (SEQ ID NO:212)
MAX_Chr8.145 Flap oligo.: 5'CCACGGACGGACGGCGTTTTT/3C6/ (SEQ ID NO:213)

FIG. 1 (cont'd)

MAX_Chr1.110 (MAX.chr1.110627198-110627213) Target DNA: (SEQ ID NO:214)

5'CTCCGCTCCCCGCAGGCCTGGCCGCGCGACGGGCACCCAGCGGGTTGTTATCAATTATTCAGGCCCCAAGTTCAC
GGGCACTGCATCCATTTCCCTCGCGTGCGCCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:215)

5'TTTCGTTTTTCGTAGGTTTGGTCGCGCGACGGGTATTTAGCGGGTTGTTATTAATTATTTAGGTTTTAAGTTTAC
GGGTATTGTATTTATTTTTTTCGCGTGCGTTT3'

PCR and Flap Assay Oligonucleotides:

MAX_Chr1.110 Forward Primer: 5'TTTCGTAGGTTTGGTCGCG3' (SEQ ID NO:216)
MAX_Chr1.110 Reverse Primer: 5'AACCTAAATAATTAATAACAACCCGC3' (SEQ ID NO:217)
MAX_Chr1.110 Flap oligo.: 5'CCACGGACGGCGACGGGTATT/3C6/(SEQ ID NO:218)

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FIG. 1 (cont'd)

NFIX Target DNA: (SEQ ID NO:219)

5'GTGGGCCGGGCGTGACGCGCGGTCAAAGTGCAATGATTTTTCAGTTCGGTTGGCTAAACAGGGTCAGAGCTGAGA
GCGAAGCAGAAGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:220)

5'GTGGGTCGGGCGTGACGCGCGGTTAAAGTGTAATGATTTTTTAGTTCGGTTGGTTAAATAGGGTTAGAGTTGAGA
GCGAAGTAGAAGG3'

PCR and Flap Assay Oligonucleotides:

NFIX_HM Forward Primer: 5'TGGTTCGGGCGTGACGCG3' (SEQ ID NO:221)
NFIX_HM Reverse Primer: 5'TCTAACCCTATTTAACCAACCGA3' (SEQ ID NO:222)
NFIX_HM Flap oligo.: 5'CGCCGAGGGCGGTTAAAGTG/3C6/ (SEQ ID NO:223)

FIG. 1 (cont'd)

NKX2-6 Target DNA: (SEQ ID NO:224)

5'GGACCTCCTCGGCCCCGCCCCATCCGCCTTCGGGATGCTGCTGAGCCCCGTCACCTCCACCCCCTTCTCGGTCAA
GGACATCCTGCGACTGGAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:225)

5'GGATTTTTTCGGTTTCGTTTTATTCGTTTTCGGGATGTTGTTGAGTTTCGTTATTTTTATTTTTTTTTCGGTTAA
GGATATTTTGCGATTGGAG3'

PCR and Flap Assay Oligonucleotides:

NKX2-6 Forward Primer: 5'GATTTTTTCGGTTTCGTTTTATTCG3' (SEQ ID NO:226)
NKX2-6 Reverse Primer: 5'CAATCGCAAAATATCCTTAACCGA3' (SEQ ID NO:227)
NKX2-6 Flap oligo.: 5'CCACGGACGGTTTTCGGGATG/3C6/ (SEQ ID NO:228)

FIG. 1 (cont'd)

OPLAH Target DNA: (SEQ ID NO:229)

5'CTGTCAGTGCTGACCGAGCGCCGCGCCTTCCGGCCATACGGGCTCCACGGTGCGCGGTTCCCCAGCCCTCGCGGC
CCTCCCCGCCCCCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:230)

5'TTGTTAGTGTTGATCGAGCGTCGCGTTTTTCGGTTATACGGGTTTTACGGTGCGCGGTTTTTTAGTTTTCGCGGT
TTTTTTCGTTTTCG3'

PCR and Flap Assay Oligonucleotides:

OPLAH Forward Primer:	5'CGTCGCGTTTTTCGGTTATACG3' (SEQ ID NO:231)
OPLAH Reverse Primer:	5'CGCGAAAACTAAAAAACCGCG3' (SEQ ID NO:232)
OPLAH Flap oligo.:	5'CCACGGACGGCACCGTAAAAC/3C6/ (SEQ ID NO:233)

FIG. 1 (cont'd)

PARP15 Target DNA: (SEQ ID NO:234)

5'CGGAGTATGGTGAGGAGCGCGGGGGACGGGTGCGGGAAGGGGACAGCAGGGCTGAGCCTGGGGCCCGCAAGACCC
AGCAGCCCGAGCGGGCGCAGAGACCCCACGCCACGCACA3'

Bisulfite-converted Target DNA: (SEQ ID NO:235)

5'CGGAGTATGGTGAGGAGCGCGGGGGACGGGTGCGGGAAGGGGATAGTAGGGTTGAGTTTGGGGTTCGTAAGATTT
AGTAGTTCGAGCGGGCGTAGAGATTTTACGTTACGTATA3'

PCR and Flap Assay Oligonucleotides:

PARP15 Forward Primer:	5'GGTTGAGTTTGGGGTTCG3' (SEQ ID NO:236)
PARP15 Reverse Primer:	5'CGTAACGTAAAATCTCTACGCCC3' (SEQ ID NO:237)
PARP15 Flap oligo.:	5'CCACGGACGCGCTCGAACTAC/3C6/ (SEQ ID NO:238)

FIG. 1 (cont'd)

PRDM14 Target DNA: (SEQ ID NO:239)

5'GGAGAGCAGCCCGCAGAACCTGGCCGCGTACTACACGCCTTTCCCGTCCTATGGACACTACAGAAACAGCCTGGC
CACCGTGGAGGAAGACTTCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:240)

5'GGAGAGTAGTTCGTAGAATTTGGTCGCGTATTATACGTTTTTTTCGTTTTATGGATATTATAGAAATAGTTTGGT
TATCGTGGAGGAAGATTTTT3'

PCR and Flap Assay Oligonucleotides:

PRDM14 Forward Primer: 5'GAGTAGTTCGTAGAATTTGGTCG3' (SEQ ID NO:241)
PRDM14 Reverse Primer v2: 5'CCACGATAACCAAACTATTTCTATAATATCC3' (SEQ ID NO:242)
PRDM14 Flap oligo.: 5'CCACGGACGGCGTATTATACG/3C6/(SEQ ID NO:243)

PRDM14 Forward Primer v3: 5'GGAGAGTAGTTCGTAGAATTTGG3' (SEQ ID NO:244)
PRDM14 Rev. Primer v3: 5'CTATTTCTATAATATCCATAAAACGAAAAAAACGT3'(SEQ ID NO:245)
PRDM14 Flap oligo. v3: 5'CCACGGACGGTCGCGTATTAT/3C6/ (SEQ ID NO:246)

FIG. 1 (cont'd)

PRKCB_28 Target DNA: (SEQ ID NO:247)

5'GGGAAGGTGCCCTGCGCGCGCGCGCTCACCAGATGAAGTCGGTGCAGTGGCTGCAGAAGGTGGGCTGCTTGAAGA
AGCGGGCGGTGAATTTG3'

Bisulfite-converted Target DNA: (SEQ ID NO:248)

5'GGGAAGGTGTTTTGCGCGCGCGCGTTTATTAGATGAAGTCGGTGTAGTGGTTGTAGAAGGTGGGTTGTTTGAAGA
AGCGGGCGGTGAATTTG3'

PCR and Flap Assay Oligonucleotides:

PRKCB_28 Forward Primer: 5'GGAAGGTGTTTTGCGCG3' (SEQ ID NO:249)
PRKCB_28 Reverse Primer: 5'CTTCTACAACCACTACACCGA3' (SEQ ID NO:250)
PRKCB_28 Flap oligo.: 5'CCACGGACGGCGCGCGTTTAT/3C6/ (SEQ ID NO:251)

FIG. 1 (cont'd)

PTGDR Target DNA: (SEQ ID NO:252)

5'GCCTCGGGGCCCGGGGACTCACAATTACGGGCAGAGAACACATAGTGAAGAGCACGGTCATCAGCGCCAGCAGCA
GGAGGTGATCCAGCTCCTCCAGGGGCTGAGGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:253)

5'GTTTCGGGGTTCGGGGATTTATAATTACGGGTAGAGAATATATAGTGAAGAGTACGGTTATTAGCGTTAGTAGTA
GGAGGTGATTTAGTTTTTTTAGGGGTTGAGGG3'

PCR and Flap Assay Oligonucleotides:

PTGDR Forward Primer:	5'GGGTTCGGGGATTTATAATTACGG3' (SEQ ID NO:254)
PTGDR Reverse Primer:	5'CCTCCTACTACTAACGCTAATAACC3' (SEQ ID NO:255)
PTGDR Flap oligo.:	5'CCACGGACGCGTACTCTTCAC/3C6/ (SEQ ID NO:256)

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FIG. 1 (cont'd)

PTGDR_9 Target DNA: (SEQ ID NO:257)

5'GGCGGCTGCAGCGGCACCCGCGCTCCTGCACCAGGGACTGTGCCGAGCCGCGCGCGGACGGGAGGGAAGCGTCCC
CTCAG

Bisulfite-converted Target DNA: (SEQ ID NO:258)

5'GGCGGTTGTAGCGGTATTCGCGTTTTTGTATTAGGGATTGTGTCGAGTCGCGCGCGGACGGGAG*G*GAAGCGTTTT
TTTAG

PCR and Flap Assay Oligonucleotides:

PTGDR_9 Forward Primer: 5'GTTGTAGCGGTATTCGCG3' (SEQ ID NO:259)
PTGDR_9 Reverse Primer: 5'CTTCTCTCCCGTCCGCGC3' (SEQ ID NO:260)
PTGDR_9 Flap oligo.: 5'CGCCGAGGCGCGACTCGACA/3C6/ (SEQ ID NO:261)

FIG. 1 (cont'd)

Human RASSF1 Target DNA: (SEQ ID NO:262)

5'TCCAGAAACACGGGTATCTCCGCGTGGTGCTTTGCGGTCGCCGTCGTTGTGGCCGTC**CG**GGGTGGGGTGTGAGGA
GGGGACGAAGGAGGGAAGGAAGGGCAAGGCGGGGGGGGCTCTGCGAGAGCGCGCCCAGCCCCGCCTTC3'

Bisulfite-converted Target DNA: (SEQ ID NO:263)

5'TTTAGAAATACGGGTATTTTCGCGTGGTGTTTTGCGGTCGTCGTCGTTGTGGTCGTT**CG**GGGTGGGGTGTGAGGA
GGGGACGAAGGAGGGAAGGAAGGGTAAGGCGGGGGGGGTTTTGCGAGAGCGCGTTTAGTTTCGTTTTT3'

PCR and Flap Assay Oligonucleotides:

Human RASSF1 Forward Primer v2: 5'AGAAATACGGGTATTTTCGCG3' (SEQ ID NO:264)
Human RASSF1 Reverse Primer v2: 5'CCACAACGACGACGACC3'(SEQ ID NO:265)
Human RASSF1 Flap oligo. V2: 5'CCACGGACGCGCAAAACACCA/3C6/(SEQ ID NO:266)

FIG. 1 (cont'd)

SHOX2 Target DNA: (SEQ ID NO:267)

5′CGGTCGGGCAGGCGGGACGGAGATTACCTGGCTGTCCAGGGGACCTTATGCAGGGTTTGGCCCGAGCCCAGGGGC
AGCGAGGGGCGTCTGCGGATGCGGCTCCCTGTGCGGCACAACACC3′

Bisulfite-converted Target DNA: (SEQ ID NO:268)

5′CGGTCGGGTAGGCGGGACGGAGATTATTTGGTTGTTTAGGGGATTTTATGTAGGGTTTGGTTCGAGTTTAGGGGT
AGCGAGGGGCGTTTGCGGATGCGGTTTTTTGTGCGGTATAATATT3′

PCR and Flap Assay Oligonucleotides:

SHOX2 Forward Primer:	5′GTTCGAGTTTAGGGGTAGCG3′ (SEQ ID NO:269)
SHOX2 Reverse Primer:	5′CCGCACAAAAAACCGCA3′ (SEQ ID NO:270)
SHOX2 Flap oligo.:	5′CCACGGACGATCCGCAAACGC/3C6/ (SEQ ID NO:271)

FIG. 1 (cont'd)

SHROOM1 Target DNA: (SEQ ID NO:272)

5'CCGGAGCACTCGCCGCTGCGCGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:273)

5'TCGGAGTATTCGTCGTTGCGCGTTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAG

PCR and Flap Assay Oligonucleotides:

SHROOM1_HM Forward Primer:	5'GGAGTATTCGTCGTTGCG3' (SEQ ID NO:274)
SHROOM1_HM Reverse Primer:	5'CGAAAACCGCCTACCGC3' (SEQ ID NO:275)
SHROOM1_HM Flap oligo.:	5'CGCCGAGGGCGTTTTGAAGT/3C6/ (SEQ ID NO:276)

FIG. 1 (cont'd)

SKI Target DNA: (SEQ ID NO:277)

5'CCCGGGCCTACGGTCCTCCCGCCACCTCCACGGGGCGGCTGTTGGGGCCCCACCAGGCAGAGCCGTGTTCTCAGG
CGTTGGCTCTCATGGAGGTGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:278)

5'TTCGGGTTTACGGTTTTTTCGTTATTTTTACGGGGCGGTTGTTGGGGTTTTATTAGGTAGAGTCGTGTTTTTAGG
CGTTGGTTTTTATGGAGGTGG3'

PCR and Flap Assay Oligonucleotides:

SKI Forward Primer: 5'ACGGTTTTTTCGTTATTTTTACGGG3' (SEQ ID NO:279)
SKI Reverse Primer: 5'CAACGCCTAAAAACACGACTC3' (SEQ ID NO:280)
SKI Flap oligo.: 5'CGCCGAGGGGCGGTTGTTGG/3C6/ (SEQ ID NO:281)

FIG. 1 (cont'd)

S1PR4 Target DNA: (SEQ ID NO:282)

5'GGGCCTGTCCCGTTCCCTGCTCCCCATACAGGCGAGGCTGCGTGCACACAGCTTCCTGTACCCCAGGAGGGCCTG
CCTGGCACGCACCCGGTGGCTGCACCATCCACACGCAAGACTGCAACTTCAGATGCTCCGCACGCTGGAGATG3'

Bisulfite-converted Target DNA: (SEQ ID NO:283)

5'GGGTTTGTTTCGTTTTTTGTTTTTTATATAGGCGAGGTTGCGTGTATATAGTTTTTTGTATTTTAGGAGGGTTTG
TTTGGTACGTATTCGGTGGTTGTATTATTTATACGTAAGATTGTAATTTTAGATGTTTCGTACGTTGGAGATG3'

PCR and Flap Assay Oligonucleotides:

S1PR4_HM Forward Primer: 5'TTATATAGGCGAGGTTGCGT3' (SEQ ID NO:284)
S1PR4_HM Reverse Primer: 5'CTTACGTATAAATAATACAACCACCGAATA3' (SEQ ID NO:285)
S1PR4_HM Flap oligo.: 5'CCACGGACGACGTACCAAACA/3C6/(SEQ ID NO:286)

FIG. 1 (cont'd)

SLC12A8 Target DNA: (SEQ ID NO:287)

5′CGGAGCTAGGAGGGTGGGGCTCGGAGGGCGCAGGAAGAGCGGCTCTGCGAGGAAAGGGAAAGGAGAGGCCGCTTC
TGGGAAGGGACCC3′

Bisulfite-converted Target DNA: (SEQ ID NO:288)

5′CGGAGTTAGGAGGGTGGGGTTCGGAGGGCGTAGGAAGAGCGGTTTTGCGAGGAAAGGGAAAGGAGAGGTCGTTTT
TGGGAAGGGATTT3′

PCR and Flap Assay Oligonucleotides:

SLC12A8 Forward Primer:	5′TTAGGAGGGTGGGGTTCG3′ (SEQ ID NO:289)
SLC12A8 Reverse Primer:	5′CTTTCCTCGCAAAACCGC3′ (SEQ ID NO:290)
SLC12A8 Flap oligo.:	5′CCACGGACGGGAGGGCGTAGG/3C6/ (SEQ ID NO:291)

FIG. 1 (cont'd)

SOBP Target DNA: (SEQ ID NO:292)

5'GCCCCGGCGGGCCCCGAGGCGGCCGCGGCCTGCAACGTCATCGTGAACGGCACGCGCGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:293)

5'GTTTCGGCGGGTTTCGAGGCGGTCGCGGTTTGTAACGTTATCGTGAACGGTACGCGCGG3'

PCR and Flap Assay Oligonucleotides:

SOBP_HM Forward Primer: 5'TTTCGGCGGGTTTCGAG3' (SEQ ID NO:294)
SOBP_HM Reverse Primer: 5'CGTACCGTTCACGATAACGT3' (SEQ ID NO:295)
SOBP_HM Flap oligo.: 5'CGCCGAGGGGCGGTCGCGGT/3C6/ (SEQ ID NO:296)
SOBP_HM Flap oligo. v2: 5'CGCCGAGGTTACAAACCGCG/3C6/ (SEQ ID NO:297)
SOBP_HM Flap oligo. V3: 5'CGCGCCGAGGTTACAAACCGCGACCG/3C6/ (SEQ ID NO:431)

FIG. 1 (cont'd)

SPOCK2 Target DNA: (SEQ ID NO:298)

5'CTAGGCGAGATGGTGGAAGGCGTGTCCGTACGGGGGTGGGCTGGGGTCCCCGTGCAGAAGGGCGCGCGAGGACCC
AGGCTGGTTTTCCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:299)

5'TTAGGCGAGATGGTGGAAGGCGTGTTCGTACGGGGGTGGGTTGGGGTTTTCGTGTAGAAGGGCGCGCGAGGATTT
AGGTTGGTTTTTTT3'

PCR and Flap Assay Oligonucleotides:

SPOCK2 Forward Primer: 5'CGAGATGGTGGAAGGCG3' (SEQ ID NO:300)
SPOCK2 Reverse Primer: 5'GCGCCCTTCTACACGAA3' (SEQ ID NO:301)
SPOCK2 Flap oligo.: 5'CCACGGACGGTGTTCGTACGG/3C6/ (SEQ ID NO:302)

FIG. 1 (cont'd)

ST8SIA1 Target DNA: (SEQ ID NO:303)

5′GCGCTGCTGCGCCGCCAGGCAAGGCGAGGGTCCGGGAGAAGGCTCGGCTCCCTCCTAAACATGTGGCCCGTGGCG
TCCCCTTGTCCCCTCCGAGCGATGCTCCTGCGCCCTTCGCCGCCTCCCGCGCTGCTGCGCCGCCAGGCAA3′

Bisulfite-converted Target DNA: (SEQ ID NO:304)

5′GGCGAGGGTTCGGGAGAAGGTTCGGTTTTTTTTTAAATATGTGGTTCGTGGCGTTTTTTTGTTTTTTTCGAGCGA
TGTTTTTGCGTTTTTCGTCGTTTTTCGCGTTGTTGCGTCGTTAGGTAA3′

PCR and Flap Assay Oligonucleotides:

ST8SIA1 Forward Primer: 5′AAATATGTGGTTCGTGGCGTT3′ (SEQ ID NO:305)
ST8SIA1 Reverse Primer: 5′ACGCAACAACGCGAAAAAC3′ (SEQ ID NO:306)
ST8SIA1 Flap oligo.: 5′CGCCGAGGCGACGAAAAACG/3C6/ (SEQ ID NO:307)

FIG. 1 (cont'd)

ST8SIA1_22 Target DNA: (SEQ ID NO:308)

5'ACGAGAAAGAGATCGTGCAGGGGGTGCTGCAACAGGGCACGGCGTGGAGGAGGAACCAGACCGCGGCCAGAGCGT
TCAGGTACTCCTGCCCTCGCGGCTCCTCCCCTCTAGCGTCCTTTCCTCCCCGAGTGCAGAGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:309)

5'ACGAGAAAGAGATCGTGTAGGGGGTGTTGTAATAGGGTACGGCGTGGAGGAGGAATTAGATCGCGGTTAGAGCGT
TTAGGTATTTTTGTTTTCGCGGTTTTTTTTTTTTAGCGTTTTTTTTTTTTCGAGTGTAGAGG3'

PCR and Flap Assay Oligonucleotides:

ST8SIA1_22 Forward Primer: 5'GGGGTGTTGTAATAGGGTACG3' (SEQ ID NO:310)
ST8SIA1_22 Reverse Primer: 5'CTAAACGCTCTAACCGCGA3' (SEQ ID NO:311)
ST8SIA1_22 Flap oligo.: 5'CCACGGACGGGCGTGGAGGAG/3C6/ (SEQ ID NO:312)

FIG. 1 (cont'd)

SP9 Target DNA: (SEQ ID NO:313)

5'CGCGCCGTTGGTCACCTCGCCGGCCGCCAGCGTCGAATGGAAGCCCGACTTGTACCAGGACTCGTACGGGTGCGC
CATGCCCACGCGCGGGTACAGCCCGTCGGCTGCCGTCGTGTG3'

Bisulfite-converted Target DNA: (SEQ ID NO:314)

5'CGCGTCGTTGGTTATTTCGTCGGTCGTTAGCGTCGAATGGAAGTTCGATTTGTATTAGGATTCGTACGGGTGCGT
TATGTTTACGCGCGGGTATAGTTCGTCGGTTGTCGTCGTGTG3'

PCR and Flap Assay Oligonucleotides:

SP9 Forward Primer: 5'TAGCGTCGAATGGAAGTTCGA3' (SEQ ID NO:315)
SP9 Reverse Primer: 5'GCGCGTAAACATAACGCACC3' (SEQ ID NO:317)
SP9 Flap oligo.: `5'CCACGGACGCCGTACGAATCC/3C6/ (SEQ ID NO:318)

SP9 Forward Primer Universal: 5'GGTCGTTAGCGTCGAATG3' (SEQ ID NO:316)
SP9 Reverse Primer: 5'GCGCGTAAACATAACGCACC3' (SEQ ID NO:317)
SP9 Flap oligo.: 5'CCACGGACGCCGTACGAATCC/3C6/ (SEQ ID NO:318)

FIG. 1 (cont'd)

SUCLG2 Target DNA: (SEQ ID NO:319)

5'GGTTCCTTCCCGTGGGTTCTTAATCGTCTCGCTGACTTCCAGAATGAAACTGCAGACCCTCGCGGTAAAGATGGC
GTGACCAGAA3'

Bisulfite-converted Target DNA: (SEQ ID NO:320)

5'GGTTTTTTTTCGTGGGTTTTTAATCGTTTCGTTGATTTTTAGAATGAAATTGTAGATTTTCGCGGTAAAGATGGC
GTGATTAGAA3'

PCR and Flap Assay Oligonucleotides:

SUCLG2_HM Forward Primer: 5'TCGTGGGTTTTTAATCGTTTCG3' (SEQ ID NO:321)
SUCLG2_HM Reverse Primer: 5'TCACGCCATCTTTACCGC3' (SEQ ID NO:322)
SUCLG2_HM Flap oligo.: 5'CCACGGACGCGAAAATCTACA/3C6/ (SEQ ID NO:323)

SUCLG2_HM For. Primer Univ.: 5'GGTTTTTTTTCGTGGGTTTTTAATCG3'(SEQ ID NO:324)
SUCLG2_HM Rev. Primer Univ.: 5'CTAATCACGCCATCTTTACCG3'(SEQ ID NO:325)
SUCLG2_HM Flap oligo. Univ.: 5'CCACGGACGGTTTCGTTGATT/3C6/(SEQ ID NO:326)

FIG. 1 (cont'd)

TBX15 Target DNA Region 1: (SEQ ID NO:327)

5'GGAGTGAGTGCCTACAACGCGCAGGCCGGACTGATCCCCCGTTGCTGCAGGTTGGTGCCCCAAGCTGCGGGTGCT
CGGGCGCCAACTAAAGCCAGCTCTGTCCAGACGCGGAAAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:328)

5'GGAGTGAGTGTTTATAACGCGTAGGTCGGATTGATTTTTCGTTGTTGTAGGTTGGTGTTTTAAGTTGCGGGTGTT
CGGGCGTTAATTAAAGTTAGTTTTGTTTAGACGCGGAAAG3'

PCR and Flap Assay Oligonucleotides:

TBX15 Forward Primer: 5'CGTAGGTCGGATTGATTTTTCGT3' (SEQ ID NO:329)
TBX15 Reverse Primer: 5'TCTAAACAAAACTAACTTTAATTAACGCCC3' (SEQ ID NO:330)
TBX15 Flap oligo.: 5'CCACGGACGCGAACACCCGCA/3C6/ (SEQ ID NO:331)

FIG. 1 (cont'd)

TBX15 Target DNA Region 2: (SEQ ID NO:403)

5'GGAAGGAAATTGCGGGTTCCCGTCTGCCTTGTCTCCAGCTTCTCTGCTGAAGCCCGGTAGCAGTGAATGCGCGCT
GACTTTCAGCGACGACTCCTGGAAGCAACGCCA3'

Bisulfite-converted Target DNA: (SEQ ID NO:404)

5'GGAAGGAAATTGCGGGTTTTCGTTTGTTTTGTTTTTAGTTTTTTTGTTGAAGTTCGGTAGTAGTGAATGCGCGTT
GATTTTTAGCGACGATTTTTGGAAGTAACGTTA3'

PCR and Flap Assay Oligonucleotides:

TBX15 Reg. 2 Forward Primer: 5' AGGAAATTGCGGGTTTTCG3' (SEQ ID NO:332)
TBX15 Reg. 2 Forward Primer Univ.:5'GGAAGGAAATTGCGGGTTTTC3' (SEQ ID NO:333)
TBX15 Reg. 2 Reverse Primer: 5'CCAAAAATCGTCGCTAAAAATCAAC3' (SEQ ID NO:334)
TBX15 Reg. 2 Flap oligo.: 5'CCACGGACGCGCGCATTCACT/3C6/ (SEQ ID NO:335)

FIG. 1 (cont'd)

TRH Target DNA: (SEQ ID NO:336)

5'GGCGGCCGCGACCCCTCCCCGCTGACCTCACTCGAGCCGCCGCCTGGCGCAGATATAAGCGGCGGCCCATCTGAA
GAGGGCTCGGCAGGCGCCCGGGGTC3'

Bisulfite-converted Target DNA:(SEQ ID NO:337)

5'GGCGGTCGCGATTTTTTTTCGTTGATTTTATTCGAGTCGTCGTTTGGCGTAGATATAAGCGGCGGTTTATTTGAA
GAGGGTTCGGTAGGCGTTCGGGGTT3'

PCR and Flap Assay Oligonucleotides:

TRH Forward Primer: 5'TTTCGTTGATTTTATTCGAGTCG3' (SEQ ID NO:338)
TRH Reverse Primer: 5'TCTTCAAATAAACCGCCGC3' (SEQ ID NO:339)
TRH Flap oligo.: 5'CGCCGAGGGTCGTTTGGCGT/3C6/ (SEQ ID NO:340)
TRH_ Forward Primer 2: 5'TTTTCGTTGATTTTATTCGAGTCGTC (SEQ ID NO:432)
TRH_ Reverse Primer 2: 5'GAACCCTCTTCAAATAAACCGC (SEQ ID NO:433)
TRH_ Flap oligo. 2: 5'CGCGCCGAGGCGTTTGGCGTAGATATAAGC/3C6/ (SEQ ID NO:434)

FIG. 1 (cont'd)

TSC22D4 Target DNA: (SEQ ID NO:341)

5′CGGGTGGTGAAGCTGCCCCACGGCCTGGGAGAGCCTTATCGCCGCGGTCGCTGGACGTGTGTGGATGTTTATGAG
CGAGACCTGGAGCCCCACAGCTTCGGCGGACTCCTGGAGGGAA3′

Bisulfite-converted Target DNA: (SEQ ID NO:342)

5′CGGGTGGTGAAGTTGTTTTACGGTTTGGGAGAGTTTTATCGTCGCGGTCGTTGGACGTGTGTGGATGTTTATGAG
CGAGATTTGGAGTTTTATAGTTTCGGCGGATTTTTGGAGGGAA3′

PCR and Flap Assay Oligonucleotides:

TSC22D4 Forward Primer:	5′GTTTGGGAGAGTTTTATCGTCG3′ (SEQ ID NO:343)
TSC22D4 Reverse Primer:	5′CCTCCAAAAATCCGCCGA3′ (SEQ ID NO:344)
TSC22D4 Flap oligo.:	5′CGCCGAGGGCGGTCGTTGGA/3C6/ (SEQ ID NO:345)

FIG. 1 (cont'd)

ZDHHC1-zincfinger, DHHC-typecontaining1 Target DNA: (SEQ ID NO:346)

5'GGGGCGGGGGCCGACAGCCCACGCTGGCGCGGCAGGCGCGTGCGCCCGCCGTTTTCGTGAGCCCGAGCAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:347)

5'GGGGTCGGGGTCGATAGTTTACGTTGGCGCGGTAGGCGCGTGCGTTCGTCGTTTTCGTGAGTTCGAGTAG3'

PCR and Flap Assay Oligonucleotides:

ZDHHC1 Forward Primer:	5'GTCGGGGTCGATAGTTTACG3' (SEQ ID NO:348)
ZDHHC1 Reverse Primer_v3:	5'ACTCGAACTCACGAAAACG3' (SEQ ID NO:349)
ZDHHC1 Flap oligo.:	5'CGCCGAGGGACGAACGCACG/3C6/ (SEQ ID NO:350)

FIG. 1 (cont'd)

ZMIZ1 Target DNA: (SEQ ID NO:351)

5'GGAGCCCCCAGCCCCACGCGGGCACACGCAGGGTGGGTGGTCACGCCCGCAGGGTCCGCGAGCGCGGCGCAGAGC
GCGGGCCGTGGGAAGTTTCTC3'

Bisulfite-converted Target DNA: (SEQ ID NO:352)

5'GGAGTTTTTAGTTTTACGCGGGTATACGTAGGGTGGGTGGTTACGTTCGTAGGGTTCGCGAGCGCGGCGTAGAGC
GCGGGTCGTGGGAAGTTTTTT3'

PCR and Flap Assay Oligonucleotides:

ZMIZ1 Forward Primer:	5'GTAGGGTGGGTGGTTACG3' (SEQ ID NO:353)
ZMIZ1 Reverse Primer:	5'AACTTCCCACGACCCGC3' (SEQ ID NO:354)
ZMIZ1 Flap oligo.:	5'CGCCGAGGGTTCGTAGGGTT/3C6/ (SEQ ID NO:355)

FIG. 1 (cont'd)

ZNF132 Target DNA: (SEQ ID NO:356)

5'GGCGCCGCCATTGCGGTCCTCATTTTGCTGCTGGTGGGTTGGGCTACAGCAGGCCTCTGGAGCCACACCAGGGCA
CGGGAGTGGGTGCAGGGACCGTCACCGCGCCTTCACACGCACCATAGTGCCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:357)

5'GGCGTCGTTATTGCGGTTTTTATTTTGTTGTTGGTGGGTTGGGTTATAGTAGGTTTTTGGAGTTATATTAGGGTA
CGGGAGTGGGTGTAGGGATCGTTATCGCGTTTTTATACGTATTATAGTGTTT3'

PCR and Flap Assay Oligonucleotides:

ZNF132 Forward Primer v2: 5'TGGAGTTATATTAGGGTACGGGA3' (SEQ ID NO:358)
ZNF132 Reverse Primer: 5'ACACTATAATACGTATAAAAACGCGATA3' (SEQ ID NO:359)
ZNF132 Flap oligo.: 5'CCACGGACGAACGATCCCTAC/3C6/ (SEQ ID NO:360)

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FIG. 1 (cont'd)

ZNF329 Target DNA: (SEQ ID NO:361)

5'GGCGGCGAGGGGCGCGTCCGCGGGTGGGTTTCACCTGGGTGGTGGGCATGTCGGGCCCGCTAGGGCGAGGGTCTG
GCCAGGGGCGTAGTTCTCCTGGTGGGTGGGGACGCTCCGTGGCGATTGGGGTCACTCCTCTGAGG3'

Bisulfite-converted Target DNA: (SEQ ID NO:362)

5'GGCGGCGAGGGGCGCGTTCGCGGGTGGGTTTTATTTGGGTGGTGGGTATGTCGGGTTCGTTAGGGCGAGGGTTTG
GTTAGGGGCGTAGTTTTTTTGGTGGGTGGGGACGTTTCGTGGCGATTGGGGTTATTTTTTTGAGG3'

PCR and Flap Assay Oligonucleotides:

ZNF329 Forward Primer: 5'GGTGGTGGGTATGTCGG3' (SEQ ID NO:363)
ZNF329 Reverse Primer: 5'CCAATCGCCACGAAACG3' (SEQ ID NO:364)
ZNF329 Flap oligo.: 5'CCACGGACGGGTTCGTTAGGG/3C6/ (SEQ ID NO:365)

FIG. 1 (cont'd)

ZNF671 Target DNA: (SEQ ID NO:366)

5'CCGTGGGCGCGGACAGCTGCCGGGAGCGGCAGGCGTCTCGATCGGGGACGCAGGCACTTCCGTCCCTGCAGAGCA
TCAGACGCGTCTCGGGACACTGGGGACAACATCTCCTCCGCG3'

Bisulfite-converted Target DNA: (SEQ ID NO:367)

5'TCGTGGGCGCGGATAGTTGTCGGGAGCGGTAGGCGTTTCGATCGGGGACGTAGGTATTTTCGTTTTTGTAGAGTA
TTAGACGCGTTTCGGGATATTGGGGATAATATTTTTTTCGCG3'

PCR and Flap Assay Oligonucleotides:

ZNF671 Forward Primer:	5'GTTGTCGGGAGCGGTAGG3' (SEQ ID NO:368)
ZNF671 Reverse Primer:	5'CCAATATCCCGAAACGCGTCT3' (SEQ ID NO:369)
ZNF671 Flap oligo.:	5'CCACGGACGGCGTTTCGATCG/3C6/ (SEQ ID NO:370)
ZNF671 Flap oligo.v2:	5'AGGCCACGGACGGCGGATTTATCGGGTTATAGT/3C6/ (SEQ ID NO:435)

FIG. 1 (cont'd)

ZNF781:Chr19:38183137-38183018(GRCh37/hg19) Target DNA: (SEQ ID NO:371)

5'AAGCTGCGCCCGGAGACGTGGGAGCGTTCTCTTGTTTTCCGAGTGCGCGGACTCATCGGGTCACAGTTTATGCTT
TTATGACGCGGTGAGTCCAGCCACTGATTCCTAACGGTTTAGAGT3'

Bisulfite-converted Target DNA: (SEQ ID NO:372)

5'AAGTTGCGTTCGGAGACGTGGGAGCGTTTTTTTGTTTTTCGAGTGCGCGGATTTATCGGGTTATAGTTTATGTTT
TTATGACGCGGTGAGTTTAGTTATTGATTTTTAACGGTTTAGAGT3'

PCR and Flap Assay Oligonucleotides:

ZNF781 Forward Primer:	5'CGTTTTTTTGTTTTTCGAGTGCG3' (SEQ ID NO:373)
ZNF781 Reverse Primer:	5'TCAATAACTAAACTCACCGCGTC3' (SEQ ID NO:374)
ZNF781 Flap oligo.:	5'CCACGGACGGCGGATTTATCG/3C6/ (SEQ ID NO:375)

FIG. 1 (cont'd)

FPR1 Target RNA: (SEQ ID NO:438)

5'GUUGCCAUGUUGACGGUGAGAGGCAUCAUCCGGUUCAUCAUUGGCUUCAGCGCACCCAUGUCCAUCGUUGCUGUC
AGUUAUGGGCUUAUUGCCACCAAGAUCCACA3'

PCR and Flap Assay Oligonucleotides:

FPR1 Forward Primer: 5'TGACGGTGAGAGGCATCA3' (SEQ ID NO:439)
FPR1 Reverse Primer: 5'GGTGGCAATAAGCCCATAACTG3' (SEQ ID NO:440)
FPR1 Hydrolysis Probe oligo.: 5'CGGTTCATCATTGGCTTCAGCGC (SEQ ID NO:441)

(Morris, S., *et al., Int J Cancer.*, (2018) 142:2355-2362)

FIG. 2

Adenocarcinoma marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
AC1	10	81002926	81002992	0%	1%	21%	15%	22%	8%	32%	1.31	42.51	30.95	45.31	15.74	64.67
AC2	2	73147720	73147790	1%	3%	24%	36%	50%	28%	59%	3.25	26.10	38.89	54.76	30.96	64.51
AC3	20	37435530	37435681	1%	1%	21%	6%	5%	9%	8%	1.55	33.32	9.43	7.37	15.07	12.88
AC4	19	3785837	3785923	0%	1%	19%	20%	5%	7%	56%	4.69	66.90	68.62	19.25	26.13	195.13
AC5	2	74726554	74726617	1%	4%	38%	47%	68%	31%	49%	3.10	27.46	34.21	49.28	22.18	35.41
AC6	12	25056015	25056162	0%	1%	10%	18%	25%	10%	35%	1.51	27.62	50.51	68.55	27.10	95.52
AC7	12	52400959	52401020	1%	1%	12%	19%	3%	1%	19%	1.59	24.05	36.73	5.80	2.58	36.73
AC8	1	156863477	156863554	1%	3%	24%	19%	32%	14%	13%	2.42	20.38	15.89	26.82	11.70	10.87
AC9	8	145106353	145106439	0%	2%	24%	29%	9%	25%	26%	4.89	59.66	71.50	22.47	62.77	65.19
AC10	7	27135634	27135679	0%	0%	10%	10%	7%	1%	40%	1.74	44.26	42.10	28.73	4.44	172.75
AC11	7	27135772	27135823	0%	1%	15%	21%	7%	2%	44%	4.72	91.85	132.84	43.76	10.27	270.65
AC12	8	145106742	145106827	0%	2%	26%	34%	10%	21%	14%	5.40	81.76	108.02	31.02	68.54	45.26
AC13	11	830323	830382	0%	1%	16%	9%	7%	1%	2%	3.56	60.14	31.27	24.44	3.55	5.83
AC14	3	124860573	124860665	0%	1%	12%	34%	12%	37%	39%	1.55	29.73	84.15	28.68	91.45	97.00
AC15	2	97193509	97193639	1%	1%	14%	7%	16%	13%	16%	2.04	26.57	14.00	30.21	23.54	29.84
AC16	1	65731423	65731507	0%	1%	14%	20%	7%	6%	5%	4.05	40.31	57.98	20.18	17.71	15.74
AC17	1	968477	968584	1%	1%	25%	22%	2%	12%	9%	2.07	40.71	35.38	3.80	18.61	15.35
AC18	14	101033514	101033620	0%	2%	18%	7%	40%	5%	14%	4.29	36.37	14.15	82.74	10.45	29.35
AC19	17	27467359	27467467	0%	0%	10%	7%	28%	4%	5%	2.69	61.89	42.49	170.64	26.04	31.95
AC20	8	145105570	145105675	1%	3%	30%	26%	9%	29%	23%	3.15	29.77	25.41	9.23	28.54	22.59
AC21	9	88137543	88137628	1%	1%	13%	9%	3%	7%	13%	1.79	24.24	16.71	5.68	13.54	22.96

FIG. 2 (cont'd)

Adenocarcinoma marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
AC1	10	ZMIZ1	NM_020338	+	0	174135	1	57178	zinc finger, MIZ-type containing 1
AC2	2	EMX1	NM_004097	+	0	3117	1	2016	empty spiracles homeobox 1
AC3	20	PPP1R16B	NM_001172735;NM_015568	+	0	1183;1183	1	26051	protein phosphatase 1, regulatory (inhibitor) subunit 16B
AC4	19	MATK	NM_002378;NM_139355;NM_139354	-	0	15973;578;578	1	4145	megakaryocyte-associated tyrosine kinase
AC5	2	LBX2	NM_001009812	-	0	3889	1	85474	ladybird homeobox 2
AC6	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46378;46378;-693;46378;-6	1	586	branched chain amino-acid transaminase 1, cytosolic
AC7	12	GRASP	NM_181711	+	1	212	1	160622	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein
AC8	1	PEAR1	NM_001080471	+	0	-45	1	375033	platelet endothelial aggregation receptor 1
AC9	8	OPLAH	NM_017570	-	1	9231	1	26873	5-oxoprolinase (ATP-hydrolysing)
AC10	7	HOXA1	NM_005522;NM_153620	-	0	-9;-9	1	3198	homeobox A1
AC11	7	HOXA1	NM_005522;NM_153620	-	0	-147;-147	1	3198	homeobox A1
AC12	8	OPLAH	NM_017570	-	0	8842	1	26873	5-oxoprolinase (ATP-hydrolysing)
AC13	11	CD151	NM_004357;NM_139029;NM_139030;NM_001039490	+	1	-2628;-2628;-2628;-2628	1	977	CD151 molecule (Raph blood group)
AC14	3	SLC12A8	NM_001195483;NM_024628	-	0	69670;71036	1	84561	solute carrier family 12 (potassium/chloride transporters), member 8
AC15	2	MAX.chr2.97193509-97193639	-	-	0	-	0	-	-
AC16	1	DNAJC6	NM_014787	+	0	994	1	9829	DnaJ (Hsp40) homolog, subfamily C, member 6
AC17	1	AGRN	NM_198576	+	0	12975	1	375790	agrin
AC18	14	BEGAIN	NM_001159531;NM_020836	-	0	893;2617	0	57596	brain-enriched guanylate kinase-associated homolog (rat)
AC19	17	MYO18A	NM_078471;NM_203318	-	0	40048;40048	0	399687	myosin XVIIIA
AC20	8	MAX.chr8.145105570-145105675	-	-	0	-	1	-	-
AC21	9	MAX.chr9.88137543-88137628	-	-	0	-	0	-	-

FIG. 2 (cont'd)

Adenocarcinoma marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
AC22	19	36909350	36909447	0%	1%	11%	15%	2%	8%	0%	1.86	29.45	41.75	5.10	22.88	1.28
AC23	2	96991058	96991212	0%	1%	15%	6%	4%	7%	4%	6.00	176.53	76.85	44.00	81.37	50.29
AC24	1	2165937	2166058	0%	0%	12%	17%	64%	6%	16%	1.37	45.20	63.35	232.04	20.22	59.05
AC25	19	58661757	58661861	1%	1%	20%	12%	9%	7%	4%	2.71	36.53	22.37	17.50	12.26	8.10
AC26	2	97193166	97193253	0%	1%	13%	8%	18%	12%	21%	1.71	42.20	25.79	55.43	37.48	67.24
AC27	8	145013661	145013775	0%	0%	26%	6%	32%	2%	10%	5.69	361.21	80.01	444.31	30.71	144.38
AC28	17	26699039	26699117	0%	1%	13%	14%	6%	1%	12%	10.25	110.62	119.15	55.00	11.44	105.40
AC29	19	58661880	58662026	1%	1%	15%	6%	7%	3%	4%	2.75	27.79	11.93	12.58	5.88	7.42
AC30	12	50297879	50297912	1%	2%	17%	20%	4%	7%	19%	2.26	22.36	27.00	5.77	9.22	24.93
AC31	1	32237695	32237880	1%	3%	22%	25%	44%	10%	14%	2.78	22.43	25.97	45.37	10.13	14.59
AC32	1	65731622	65731666	1%	2%	27%	35%	11%	12%	17%	2.00	31.36	39.95	13.18	14.20	19.04
AC33	16	23847586	23847684	0%	2%	15%	23%	18%	2%	26%	12.77	119.07	189.49	148.49	13.87	209.78
AC34	7	100075307	100075425	0%	2%	21%	18%	54%	15%	16%	3.87	45.55	39.16	116.74	32.72	34.94
AC35	1	44031599	44031658	0%	2%	24%	18%	21%	21%	18%	4.80	75.23	56.94	66.66	64.67	56.57
AC36	16	23847871	23847925	0%	1%	10%	11%	6%	2%	19%	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
AC37	16	23847938	23848020	0%	1%	15%	12%	10%	2%	15%	25.58	293.90	237.97	199.65	41.02	298.01
AC38	19	3785977	3786032	0%	1%	14%	24%	3%	5%	34%	5.84	54.36	93.77	12.00	19.42	135.15
AC39	19	37288523	37288615	1%	2%	19%	22%	26%	18%	30%	2.72	25.92	30.28	34.88	24.73	40.51
AC40	13	103046898	103046982	0%	0%	14%	10%	1%	1%	13%	1.60	47.29	32.21	2.55	1.96	41.48
AC41	2	25439185	25439264	0%	1%	10%	11%	31%	3%	7%	2.31	25.89	26.41	77.49	6.89	18.37
AC42	20	61638472	61638536	0%	1%	15%	11%	1%	5%	17%	5.21	56.31	41.95	4.97	19.39	63.96
AC43	1	32237893	32237998	1%	2%	19%	29%	52%	10%	27%	2.51	24.19	36.61	65.03	12.62	34.40
AC44	3	50378496	50378540	0%	1%	13%	14%	62%	6%	16%	6.77	84.98	89.94	406.60	38.31	101.31

FIG. 2 (cont'd)

Adenocarcinoma marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
AC22	19	LOC644189	NR_033748	+	0	-3088	1	644189	acyl-CoA thioesterase 4 pseudogene
AC23	2	ITPRIPL1	NM_178495;NM_001163524;NM_001008949;NM_001163523	+	0	-876;-886;-3;-10	1	150771	inositol 1,4,5-triphosphate receptor interacting protein-like 1
AC24	1	SKI	NM_003036	+	0	5804	0	6497	v-ski sarcoma viral oncogene homolog (avian)
AC25	19	ZNF329	NM_024620	-	0	391	1	79673	zinc finger protein 329
AC26	2	MAX.chr2.97193166-97193253	-	-	0	-	1	-	-
AC27	8	PLEC	NM_201381;NM_201383;NM_000445;NM_201378;NM_201380;NM_201384;NM_201382;NM_201379	-	1	5244;3031;37252;34036;11383;97;4449;14427	0	5339	plectin
AC28	17	SARM1	NM_015077	+	1	53	1	23098	sterile alpha and TIR motif containing 1
AC29	19	ZNF329	NM_024620	-	0	268	1	79673	zinc finger protein 329
AC30	12	FAIM2	NM_012306	-	0	-159	1	23017	Fas apoptotic inhibitory molecule 2
AC31	1	MAX.chr1.32237695-32237880	-	-	0	-	0	-	-
AC32	1	DNAJC6	NM_014787	+	0	1193	1	9829	DnaJ (Hsp40) homolog, subfamily C, member 6
AC33	16	PRKCB	NM_002738;NM_212535	+	1	287;287	1	5579	protein kinase C, beta
AC34	7	TSC22D4	NM_030935	-	1	1595	1	81628	TSC22 domain family, member 4
AC35	1	PTPRF	NM_002840;NM_130440	+	0	35053;35053	1	5792	protein tyrosine phosphatase, receptor type, F
AC36	16	PRKCB	NM_002738;NM_212535	+	0	572;572	1	5579	protein kinase C, beta
AC37	16	PRKCB	NM_002738;NM_212535	+	0	639;639	1	5579	protein kinase C, beta
AC38	19	MATK	NM_002378;NM_139355;NM_139354	-	0	15833;438;438	1	4145	megakaryocyte-associated tyrosine kinase
AC39	19	MAX.chr19.37288523-37288615	-	-	0	-	1	-	-
AC40	13	FGF14	NM_175929	-	0	7226	1	2259	fibroblast growth factor 14
AC41	2	MAX.chr2.25439185-25439264	-	-	0	-	1	-	-
AC42	20	BHLHE23	NM_080606	-	0	-85	1	128408	basic helix-loop-helix family, member e23
AC43	1	MAX.chr1.32237893-32237998	-	-	0	-	1	-	-
AC44	3	RASSF1	NM_170714;NM_007182;NM_170712;NM_170713	-	0	-129;-129;-2832;-3601	1	11186	Ras association (RalGDS/AF-6) domain family member 1

FIG. 2 (cont'd)

Adenocarcinoma marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
AC45	8	99439151	99439192	0%	1%	14%	18%	4%	1%	26%	1.44	31.01	40.63	8.77	1.90	57.44
AC46	19	37288426	37288510	0%	1%	15%	22%	30%	19%	27%	1.79	39.22	55.94	78.85	49.81	69.01
AC47	9	96715209	96715360	1%	3%	24%	15%	29%	10%	6%	3.21	29.13	17.97	35.67	11.96	6.87
AC48	15	65116396	65116440	0%	1%	11%	21%	5%	25%	2%	5.61	51.82	98.18	22.13	114.94	10.41
AC49	17	17627469	17627534	1%	1%	17%	7%	0%	19%	11%	0.77	21.31	8.72	0.57	23.63	14.12

FIG. 2 (cont'd)

Adenocarcinoma marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
AC45	8	KCNS2	NM_020697	+	0	-98	1	3788	potassium voltage-gated channel, delayed-rectifier, subfamily S, member 2
AC46	19	MAX.chr19.37288426-37288510	-	-	0	-	1	-	-
AC47	9	BARX1	NM_021570	-	1	2399	1	56033	BARX homeobox 1
AC48	15	PIF1	NM_025049	-	1	1442	1	80119	PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae)
AC49	17	RAI1	NM_030665	+	1	42683	1	10743	retinoic acid induced 1

FIG. 3

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC1	16	23847871	23847925	0%	1%	10%	11%	6%	2%	19%	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!	#DIV/0!
LC2	5	169064343	169064446	0%	1%	10%	11%	40%	2%	13%	29.90	255.47	295.59	1054.27	55.31	339.16
LC3	5	42992328	42992393	0%	4%	27%	38%	43%	23%	50%	29.14	179.62	252.75	288.47	156.43	334.25
LC4	7	37488072	37488105	0%	0%	3%	19%	6%	1%	16%	5.21	36.35	238.74	72.25	9.26	203.41
LC5	16	23847938	23848020	0%	1%	15%	12%	10%	2%	15%	25.58	293.90	237.97	199.65	41.02	298.01
LC6	19	17346461	17346549	0%	2%	9%	21%	0%	14%	26%	17.37	84.73	202.32	0.58	132.88	247.58
LC7	16	23847586	23847684	0%	2%	15%	23%	18%	2%	26%	12.77	119.07	189.49	148.49	13.87	209.78
LC8	5	42995328	42995393	0%	4%	12%	28%	50%	30%	55%	24.55	77.50	185.42	325.38	198.95	362.13
LC9	19	58238816	58238942	0%	1%	8%	15%	24%	17%	33%	8.09	94.63	175.66	277.57	203.93	390.90
LC10	7	37488118	37488163	0%	0%	2%	11%	2%	0%	12%	2.85	29.53	155.86	33.00	6.18	159.79
LC11	5	42995477	42995528	0%	5%	20%	35%	60%	39%	62%	18.40	82.13	141.62	245.53	158.96	254.67
LC12	1	110627264	110627325	0%	1%	5%	28%	52%	8%	31%	3.49	23.92	137.94	259.29	39.55	152.65
LC13	19	58011421	58011488	0%	1%	3%	22%	12%	6%	0%	3.89	17.87	129.28	71.80	35.25	1.25
LC14	12	49484143	49484184	0%	1%	5%	15%	5%	2%	9%	6.92	36.99	121.11	43.30	15.20	72.91
LC15	17	26699039	26699117	0%	1%	13%	14%	6%	1%	12%	10.25	110.62	119.15	55.00	11.44	105.40
LC16	20	47443870	47443961	0%	1%	6%	10%	4%	2%	9%	8.85	64.06	110.72	46.66	17.90	96.24
LC17	17	46675164	46675237	0%	1%	8%	20%	6%	7%	11%	4.38	43.34	109.94	35.52	37.61	58.29
LC18	8	145106742	145106827	0%	2%	26%	34%	10%	21%	14%	5.40	81.76	108.02	31.02	68.54	45.26
LC19	17	75447560	75447708	0%	0%	2%	15%	2%	0%	5%	1.28	17.71	107.93	17.41	2.23	37.60
LC20	4	102711879	102711959	0%	2%	8%	23%	12%	11%	19%	8.25	32.43	99.18	50.15	48.17	80.58
LC21	11	14926886	14926955	0%	3%	6%	29%	78%	21%	20%	8.70	18.97	99.05	266.28	70.91	68.97
LC22	17	42287927	42287988	0%	0%	11%	15%	26%	18%	32%	1.27	71.79	95.09	167.27	117.62	206.97
LC23	19	3785977	3786032	0%	1%	14%	24%	3%	5%	34%	5.84	54.36	93.77	12.00	19.42	135.15
LC24	2	182322274	182322403	0%	1%	9%	11%	10%	2%	8%	7.64	78.53	92.41	84.05	18.74	72.85

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC1	16	PRKCB	NM_002738;NM_212535	+	0	572;572	1	5579	protein kinase C, beta
LC2	5	DOCK2	NM_004946	+	1	93	1	1794	dedicator of cytokinesis 2
LC3	5	MAX.chr5.42992328-42992393	-	-	0	-	1	-	-
LC4	7	ELMO1	NM_014800	-	0	439	1	9844	engulfment and cell motility 1
LC5	16	PRKCB	NM_002738;NM_212535	+	0	639;639	1	5579	protein kinase C, beta
LC6	19	NR2F6	NM_005234	-	1	9690	1	2063	nuclear receptor subfamily 2, group F, member 6
LC7	16	PRKCB	NM_002738;NM_212535	+	1	287;287	1	5579	protein kinase C, beta
LC8	5	MAX.chr5.42995328-42995393	-	-	0	-	1	-	-
LC9	19	ZNF671	NM_024833	-	1	179	1	79891	zinc finger protein 671
LC10	7	ELMO1	NM_014800	-	0	393	1	9844	engulfment and cell motility 1
LC11	5	MAX.chr5.42995477-42995528	-	-	0	-	0	-	-
LC12	1	MAX.chr1.110627264-110627325	-	-	0	-	1	-	-
LC13	19	ZNF773	NM_198542	+	1	113	1	374928	zinc finger protein 773
LC14	12	DHH	NM_021044	-	1	4459	1	50846	desert hedgehog
LC15	17	SARM1	NM_015077	+	1	53	1	23098	sterile alpha and TIR motif containing 1
LC16	20	PREX1	NM_020820	-	0	550	1	57580	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1
LC17	17	HOXB5	NM_002147	-	1	-4061	1	3215	homeobox B5
LC18	8	OPLAH	NM_017570	-	0	8842	1	26873	5-oxoprolinase (ATP-hydrolysing)
LC19	17	sep9	NM_006640;NM_001113494;NM_001113496;NM_001113492;NM_001113493;NM_001113491	+	0	131964;75396;948;163588;78289;170069	1	10801	septin 9
LC20	4	BANK1	NM_017935;NM_001127507	+	1	116;116	1	55024	B-cell scaffold protein with ankyrin repeats 1
LC21	11	MAX.chr11.14926886-14926955	-	-	0	-	1	-	-
LC22	17	UBTF	NM_001076684;NM_001076683;NM_014233	-	0	8997;10323;7737	1	7343	upstream binding transcription factor, RNA polymerase I
LC23	19	MATK	NM_002378;NM_139355;NM_139354	-	0	15833;438;438	1	4145	megakaryocyte-associated tyrosine kinase
LC24	2	ITGA4	NM_000885	+	1	656	1	3676	integrin, alpha 4 (antigen CD49D, alpha 4 subunit of VLA-4 receptor)

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC25	3	50378496	50378540	0%	1%	13%	14%	62%	6%	16%	6.77	84.98	89.94	406.60	38.31	101.31
LC26	5	157098339	157098381	0%	1%	9%	18%	11%	2%	13%	5.53	45.71	89.03	53.16	11.52	64.27
LC27	8	124173236	124173386	0%	1%	11%	15%	49%	14%	29%	7.50	63.79	86.89	276.90	78.49	166.63
LC28	4	42153564	42153601	0%	0%	5%	13%	6%	1%	10%	2.47	36.03	85.84	37.84	7.67	70.13
LC29	19	17346401	17346450	0%	1%	5%	15%	2%	6%	14%	5.69	25.74	84.33	13.37	34.47	78.56
LC30	3	124860573	124860665	0%	1%	12%	34%	12%	37%	39%	1.55	29.73	84.15	28.68	91.45	97.00
LC31	7	37488225	37488303	0%	0%	4%	15%	4%	1%	13%	2.22	22.77	82.00	24.55	6.84	70.81
LC32	12	25055873	25055997	0%	1%	10%	22%	29%	12%	40%	4.32	36.00	77.51	103.02	44.17	140.87
LC33	3	194118747	194118919	0%	2%	13%	26%	43%	19%	41%	6.97	38.31	74.49	125.02	55.43	117.27
LC34	8	145106353	145106439	0%	2%	24%	29%	9%	25%	26%	4.89	59.66	71.50	22.47	62.77	65.19
LC35	17	46620564	46620622	0%	1%	11%	34%	21%	12%	29%	2.90	22.12	70.90	43.06	25.10	59.41
LC36	2	173099757	173099817	0%	1%	4%	16%	7%	4%	3%	3.23	17.04	69.46	30.54	19.33	11.52
LC37	19	3785837	3785923	0%	1%	19%	20%	5%	7%	56%	4.69	66.90	68.62	19.25	26.13	195.13
LC38	7	37487776	37487893	0%	1%	3%	12%	6%	0%	11%	3.09	17.32	66.89	32.54	1.97	64.09
LC39	6	6004298	6004338	1%	5%	21%	40%	58%	34%	61%	7.31	33.74	64.23	93.83	54.50	99.42
LC40	10	22541891	22541996	0%	2%	13%	22%	49%	18%	24%	5.71	36.56	63.36	141.74	52.16	69.90
LC41	1	2165937	2166058	0%	0%	12%	17%	64%	6%	16%	1.37	45.20	63.35	232.04	20.22	59.05
LC42	1	223936868	223936997	0%	1%	7%	18%	45%	16%	28%	2.13	26.29	63.20	158.81	57.13	99.50
LC43	5	1295194	1295314	0%	1%	8%	12%	26%	2%	14%	3.69	41.84	62.47	142.53	9.07	77.02
LC44	11	128564667	128564773	0%	0%	3%	13%	7%	3%	25%	2.36	15.22	60.67	32.31	14.64	120.57
LC45	1	65731423	65731507	0%	1%	14%	20%	7%	6%	5%	4.05	40.31	57.98	20.18	17.71	15.74
LC46	7	142494769	142494904	0%	1%	7%	11%	5%	2%	23%	5.22	34.83	57.90	28.83	12.03	123.00

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC25	3	RASSF1	NM_170714;NM_007182;NM_170712;NM_170713	-	0	-129;-129;-2832;-3601	1	11186	Ras association (RalGDS/AF-6) domain family member 1
LC26	5	C5orf52	NM_001145132	+	0	-221	1	100190949	chromosome 5 open reading frame 52
LC27	8	MAX.chr8.124173236-124173386	-	-	0	-	1	-	-
LC28	4	BEND4	NM_001159547;NM_207406	-	0	1331;1331	1	389206	BEN domain containing 4
LC29	19	NR2F6	NM_005234	-	1	9750	1	2063	nuclear receptor subfamily 2, group F, member 6
LC30	3	SLC12A8	NM_001195483;NM_024628	-	0	69670;71036	1	84561	solute carrier family 12 (potassium/chloride transporters), member 8
LC31	7	ELMO1	NM_014800	-	0	286	1	9844	engulfment and cell motility 1
LC32	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46520;46520;-551;46520;136	1	586	branched chain amino-acid transaminase 1, cytosolic
LC33	3	GP5	NM_004488	-	1	1248	1	2814	glycoprotein V (platelet)
LC34	8	OPLAH	NM_017570	-	1	9231	1	26873	5-oxoprolinase (ATP-hydrolysing)
LC35	17	HOXB2	NM_002145	-	1	1829	1	3212	homeobox B2
LC36	2	MAX.chr2.173099757-173099817	-	-	0	-	1	-	-
LC37	19	MATK	NM_002378;NM_139355;NM_139354	-	0	15973;578;578	1	4145	megakaryocyte-associated tyrosine kinase
LC38	7	ELMO1	NM_014800	-	0	735	1	9844	engulfment and cell motility 1
LC39	6	NRN1	NM_016588	-	0	3335	1	51299	neuritin 1
LC40	10	MAX.chr10.22541891-22541996	-	-	0	-	1	-	-
LC41	1	SKI	NM_003036	+	0	5804	0	6497	v-ski sarcoma viral oncogene homolog (avian)
LC42	1	CAPN2	NM_001146068;NM_001748	+	0	47574;36750	1	824	calpain 2, (m/II) large subunit
LC43	5	TERT	NM_001193376;NM_198253	-	0	-32;-32	1	7015	telomerase reverse transcriptase
LC44	11	FLI1	NM_002017;NM_001167681	+	0	855;2279	1	2313	Friend leukemia virus integration 1
LC45	1	DNAJC6	NM_014787	+	0	994	1	9829	DnaJ (Hsp40) homolog, subfamily C, member 6
LC46	7	MAX.chr7.142494769-142494904	-	-	0	-	1	-	-

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC47	1	161275664	161275801	0%	0%	3%	11%	0%	6%	0%	2.20	15.83	57.68	1.19	32.84	2.04
LC48	1	32237619	32237654	1%	5%	32%	35%	60%	14%	28%	7.65	53.16	57.57	100.17	23.82	46.87
LC49	1	44031599	44031658	0%	2%	24%	18%	21%	21%	18%	4.80	75.23	56.94	66.66	64.67	56.57
LC50	20	61560692	61560749	0%	1%	6%	13%	62%	19%	28%	5.65	24.19	56.55	258.74	79.08	115.84
LC51	3	122296709	122296828	0%	3%	17%	23%	65%	25%	61%	5.87	39.97	54.78	151.39	57.49	143.28
LC52	8	145104291	145104342	0%	1%	6%	13%	3%	12%	10%	3.80	26.23	54.77	13.90	49.84	41.94
LC53	4	140201231	140201277	1%	2%	7%	34%	11%	7%	21%	3.04	10.87	54.63	18.35	12.07	33.86
LC54	4	15780145	15780191	0%	2%	5%	16%	19%	8%	19%	5.10	17.13	53.63	61.39	27.48	61.88
LC55	1	111217635	111217682	0%	1%	8%	18%	3%	4%	28%	2.24	22.91	52.55	9.59	10.61	83.04
LC56	3	124860704	124860798	0%	1%	10%	21%	9%	35%	22%	1.98	23.37	51.32	20.78	84.00	51.80
LC57	12	25056015	25056162	0%	1%	10%	18%	25%	10%	35%	1.51	27.62	50.51	68.55	27.10	95.52
LC58	17	48042562	48042606	1%	3%	24%	47%	56%	25%	44%	3.67	25.97	50.24	59.12	26.78	46.42
LC59	12	107713157	107713254	0%	2%	6%	24%	2%	3%	17%	3.60	12.28	49.38	4.01	5.33	34.97
LC60	19	37464151	37464219	0%	1%	6%	20%	19%	17%	40%	2.12	15.10	47.57	43.26	38.92	93.33
LC61	12	65218381	65218413	0%	0%	1%	11%	4%	1%	8%	1.99	6.49	47.47	17.50	5.10	35.85
LC62	10	94834101	94834171	0%	1%	4%	14%	3%	1%	9%	2.24	13.39	46.30	10.38	4.51	27.46
LC63	5	169064211	169064314	0%	1%	9%	15%	30%	2%	22%	3.71	29.12	46.14	93.41	5.35	71.09
LC64	12	4273887	4274003	0%	1%	8%	17%	9%	3%	6%	3.02	22.67	45.94	24.63	7.65	17.15
LC65	12	25055634	25055804	1%	3%	14%	27%	30%	17%	50%	5.65	23.18	44.87	51.11	28.60	83.14
LC66	3	13115009	13115073	1%	2%	46%	40%	16%	3%	42%	2.26	51.11	44.54	17.94	2.82	47.14

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC47	1	MPZ	NM_000530	-	1	4098	0	4359	myelin protein zero
LC48	1	MAX.chr1.32237619-32237654	-	-	0	-	0	-	-
LC49	1	PTPRF	NM_002840;NM_130440	+	0	35053;35053	1	5792	protein tyrosine phosphatase, receptor type, F
LC50	20	DIDO1	NM_033081;NM_001193369;NM_022105;NM_080797;NM_001193370;NM_080796	-	0	8612;-2789;8612;8612;-2789;-2789	1	11083	death inducer-obliterator 1
LC51	3	PARP15	NM_001113523	+	0	261	1	165631	poly (ADP-ribose) polymerase family, member 15
LC52	8	MAX.chr8.145104291-145104342	-	-	0	-	1	-	-
LC53	4	C4orf49	NM_032623	-	1	261	1	84709	chromosome 4 open reading frame 49
LC54	4	CD38	NM_001775	+	1	215	1	952	CD38 molecule
LC55	1	KCNA3	NM_002232	-	1	20	1	3738	potassium voltage-gated channel, shaker-related subfamily, member 3
LC56	3	SLC12A8	NM_001195483;NM_024628	-	0	69539;70905	1	84561	solute carrier family 12 (potassium/chloride transporters), member 8
LC57	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46378;46378;-693;46378;-6	1	586	branched chain amino-acid transaminase 1, cytosolic
LC58	17	DLX4	NM_138281	+	0	-3999	1	1748	distal-less homeobox 4
LC59	12	BTBD11	NM_001018072	+	1	961	1	121551	BTB (POZ) domain containing 11
LC60	19	MAX.chr19.37464151-37464219	-	-	0	-	1	-	-
LC61	12	TBC1D30	NM_015279	+	1	30	1	23329	TBC1 domain family, member 30
LC62	10	CYP26A1	NM_000783;NM_057157	+	1	455;870	1	1592	cytochrome P450, family 26, subfamily A, polypeptide 1
LC63	5	DOCK2	NM_004946	+	0	-39	0	1794	dedicator of cytokinesis 2
LC64	12	MAX.chr12.4273887-4274003	-	-	0	-	1	-	-
LC65	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46759;46759;-312;46759;375	1	586	branched chain amino-acid transaminase 1, cytosolic
LC66	3	IQSEC1	NM_001134382	-	0	-392	1	9922	IQ motif and Sec7 domain 1

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC67	20	37434670	37434793	0%	1%	7%	10%	1%	1%	10%	2.23	31.25	44.50	2.57	3.70	43.59
LC68	7	100273764	100273857	0%	0%	2%	11%	0%	9%	10%	0.72	7.67	44.09	0.23	37.98	38.77
LC69	2	118981859	118981945	1%	3%	14%	33%	42%	28%	29%	4.14	18.40	42.45	53.71	35.86	36.89
LC70	8	70981960	70982028	1%	4%	26%	36%	65%	28%	58%	5.02	30.29	41.91	74.59	32.63	66.72
LC71	20	61638221	61638352	0%	1%	7%	15%	1%	2%	18%	2.59	20.85	40.85	3.78	6.32	50.89
LC72	5	77268624	77268718	1%	2%	12%	22%	34%	7%	21%	3.72	21.82	40.13	61.10	12.77	38.23
LC73	1	65731622	65731666	1%	2%	27%	35%	11%	12%	17%	2.00	31.36	39.95	13.18	14.20	19.04
LC74	1	78511734	78511827	1%	2%	15%	32%	24%	15%	51%	2.91	18.47	39.63	29.28	18.15	62.64
LC75	7	100075307	100075425	0%	2%	21%	18%	54%	15%	16%	3.87	45.55	39.16	116.74	32.72	34.94
LC76	17	73073700	73073810	0%	0%	0%	15%	8%	17%	0%	1.26	1.07	39.05	21.72	46.29	0.42
LC77	2	73147720	73147790	1%	3%	24%	36%	50%	28%	59%	3.25	26.10	38.89	54.76	30.96	64.51
LC78	12	103352075	103352138	0%	1%	3%	10%	1%	4%	16%	2.48	12.32	38.77	3.39	15.06	62.03
LC79	10	22624410	22624553	1%	2%	7%	30%	63%	11%	58%	2.80	9.42	38.62	82.32	14.11	75.75
LC80	8	98290125	98290161	0%	1%	5%	18%	10%	5%	17%	2.98	10.47	38.51	20.42	10.93	35.54
LC81	2	66808687	66808728	1%	7%	24%	48%	74%	37%	80%	5.37	19.49	38.15	58.88	29.21	63.62
LC82	15	84748786	84748909	1%	1%	9%	19%	1%	5%	13%	2.55	16.56	36.80	1.01	8.97	25.40
LC83	12	52400959	52401020	1%	1%	12%	19%	3%	1%	19%	1.59	24.05	36.73	5.80	2.58	36.73
LC84	1	32237893	32237998	1%	2%	19%	29%	52%	10%	27%	2.51	24.19	36.61	65.03	12.62	34.40
LC85	7	27205013	27205081	1%	7%	29%	50%	83%	42%	48%	5.20	20.79	36.52	60.30	30.77	35.13
LC86	4	8859253	8859363	0%	1%	4%	14%	14%	7%	18%	3.88	9.54	36.29	35.88	18.16	46.41
LC87	20	47444641	47444682	0%	1%	7%	15%	2%	1%	5%	3.03	16.83	36.12	5.94	2.12	11.01

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC67	20	PPP1R16B	NM_001172735;NM_015568	+	0	323;323	1	26051	protein phosphatase 1, regulatory (inhibitor) subunit 16B
LC68	7	GNB2	NM_005273	+	0	2402	1	2783	guanine nucleotide binding protein (G protein), beta polypeptide 2
LC69	2	MAX.chr2.118981859-118981945	-	-	0	-	1	-	-
LC70	8	PRDM14	NM_024504	-	1	1602	1	63978	PR domain containing 14
LC71	20	BHLHE23	NM_080606	-	1	166	1	128408	basic helix-loop-helix family, member e23
LC72	5	MAX.chr5.77268624-77268718	-	-	0	-	1	-	-
LC73	1	DNAJC6	NM_014787	+	0	1193	1	9829	DnaJ (Hsp40) homolog, subfamily C, member 6
LC74	1	GIPC2	NM_017655	+	1	146	1	54810	GIPC PDZ domain containing family, member 2
LC75	7	TSC22D4	NM_030935	-	1	1595	1	81628	TSC22 domain family, member 4
LC76	17	MAX.chr17.73073700-73073810	-	-	0	-	1	-	-
LC77	2	EMX1	NM_004097	+	0	3117	1	2016	empty spiracles homeobox 1
LC78	12	ASCL1	NM_004316	+	1	624	1	429	achaete-scute complex homolog 1 (Drosophila)
LC79	10	MAX.chr10.22624410-22624553	-	-	0	-	1	-	-
LC80	8	TSPYL5	NM_033512	-	1	51	1	85453	TSPY-like 5
LC81	2	MAX.chr2.66808687-66808728	-	-	0	-	1	-	-
LC82	15	LOC648809	NR_036652	+	0	-152	1	648809	elongation factor Tu GTP-binding domain-containing protein 1 pseudogene
LC83	12	GRASP	NM_181711	+	1	212	1	160622	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein
LC84	1	MAX.chr1.32237893-32237998	-	-	0	-	1	-	-
LC85	7	HOXA9	NM_152739	-	1	136	1	3205	homeobox A9
LC86	4	MAX.chr4.8859253-8859363	-	-	0	-	1	-	-
LC87	20	PREX1	NM_020820	-	0	-221	1	57580	phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 1

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC88	19	54486055	54486134	1%	2%	11%	21%	10%	3%	34%	2.95	18.45	36.01	16.74	5.84	57.48
LC89	2	26407721	26407876	0%	1%	2%	11%	47%	18%	16%	2.39	8.36	36.01	160.15	60.97	56.07
LC90	7	27196035	27196154	1%	3%	21%	31%	37%	18%	56%	3.72	24.32	35.89	42.10	20.22	64.49
LC91	7	158937376	158937476	1%	2%	8%	20%	9%	5%	21%	3.81	14.17	35.70	16.30	8.28	37.29
LC92	12	103352229	103352268	0%	1%	2%	14%	2%	3%	25%	2.42	5.87	35.68	4.29	6.57	62.78
LC93	1	968477	968584	1%	1%	25%	22%	2%	12%	9%	2.07	40.71	35.38	3.80	18.61	15.35
LC94	7	157483370	157483425	0%	1%	9%	14%	1%	3%	23%	3.36	22.18	35.26	3.79	7.43	58.67
LC95	5	178957576	178957695	1%	2%	15%	18%	41%	17%	24%	3.97	29.20	34.94	78.81	31.81	45.24
LC96	17	46675383	46675464	0%	1%	4%	11%	3%	5%	3%	1.83	12.26	34.87	9.04	14.87	8.78
LC97	19	51831114	51831160	1%	2%	17%	20%	26%	4%	26%	4.03	29.10	34.38	43.50	6.23	44.43
LC98	2	74726554	74726617	1%	4%	38%	47%	68%	31%	49%	3.10	27.46	34.21	49.28	22.18	35.41
LC99	14	70654555	70654616	0%	1%	9%	15%	1%	5%	7%	3.08	19.47	33.99	3.28	12.22	15.29
LC100	5	134879621	134879709	1%	2%	5%	21%	40%	6%	35%	3.43	8.06	33.63	65.55	9.28	56.82
LC101	12	107715041	107715084	0%	1%	5%	11%	2%	1%	19%	2.19	16.32	33.62	5.06	2.98	57.40
LC102	14	52734489	52734592	0%	1%	7%	13%	5%	3%	24%	3.42	16.75	33.53	12.05	7.48	59.56
LC103	1	101004818	101004860	0%	1%	10%	16%	3%	2%	23%	3.10	20.44	33.24	5.47	4.02	49.05
LC104	4	13524253	13524378	1%	2%	7%	21%	28%	11%	23%	3.50	10.37	32.37	43.87	17.49	36.14
LC105	10	81002926	81002992	0%	1%	21%	15%	22%	8%	32%	1.31	42.51	30.95	45.31	15.74	64.67
LC106	11	14926627	14926716	1%	1%	5%	16%	62%	16%	11%	1.78	10.57	30.66	119.53	31.77	21.41
LC107	2	176945102	176945135	1%	2%	7%	18%	7%	7%	38%	2.93	11.98	30.46	10.98	11.55	63.00
LC108	1	248020671	248020722	1%	4%	31%	40%	63%	33%	63%	3.31	23.46	30.04	47.26	24.66	47.74
LC109	11	31820365	31820418	1%	4%	19%	39%	74%	21%	59%	3.41	14.93	30.02	57.24	16.07	45.33
LC110	17	26699373	26699456	1%	2%	14%	20%	8%	2%	8%	2.84	21.52	29.85	12.44	3.28	12.27
LC111	1	214158912	214158969	1%	4%	20%	33%	50%	18%	46%	3.39	17.95	29.64	45.85	16.83	41.54

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC88	19	CACNG8	NM_031895	+	1	19766	1	59283	calcium channel, voltage-dependent, gamma subunit 8
LC89	2	FAM59B	NM_001191033;NM_001168241	+	1	4137;11762	1	150946	family with sequence similarity 59, member B
LC90	7	HOXA7	NM_006896	-	1	261	1	3204	homeobox A7
LC91	7	VIPR2	NM_003382	-	0	273	1	7434	vasoactive intestinal peptide receptor 2
LC92	12	ASCL1	NM_004316	+	1	778	1	429	achaete-scute complex homolog 1 (Drosophila)
LC93	1	AGRN	NM_198576	+	0	12975	1	375790	agrin
LC94	7	PTPRN2	NM_130842;NM_002847;NM_130843	-	0	897112;897112;897112	1	5799	protein tyrosine phosphatase, receptor type, N polypeptide 2
LC95	5	MAX.chr5.178957576-178957695	-	-	0	-	1	-	-
LC96	17	HOXB5	NM_002147	-	1	-4280	1	3215	homeobox B5
LC97	19	IGLON5	NM_001101372	+	1	16013	1	402665	IgLON family member 5
LC98	2	LBX2	NM_001009812	-	0	3889	1	85474	ladybird homeobox 2
LC99	14	SLC8A3	NM_183002;NM_058240;NM_182932;NM_033262	-	0	1232;1232;1232;1232	0	6547	solute carrier family 8 (sodium/calcium exchanger), member 3
LC100	5	MAX.chr5.134879621-134879709	-	-	0	-	1	-	-
LC101	12	BTBD11	NM_001018072	+	0	2845	0	121551	BTB (POZ) domain containing 11
LC102	14	PTGDR	NM_000953	+	1	59	1	5729	prostaglandin D2 receptor (DP)
LC103	1	GPR88	NM_022049	+	1	1091	1	54112	G protein-coupled receptor 88
LC104	4	MAX.chr4.13524253-13524378	-	-	0	-	1	-	-
LC105	10	ZMIZ1	NM_020338	+	0	174135	1	57178	zinc finger, MIZ-type containing 1
LC106	11	MAX.chr11.14926627-14926716	-	-	0	-	1	-	-
LC107	2	EVX2	NM_001080458	-	1	3588	1	344191	even-skipped homeobox 2
LC108	1	TRIM58	NM_015431	+	1	171	1	25893	tripartite motif-containing 58
LC109	11	PAX6	NM_000280;NM_001604;NM_001127612	-	0	12514;12514;19144	1	5080	paired box 6
LC110	17	SARM1	NM_015077	+	1	387	1	23098	sterile alpha and TIR motif containing 1
LC111	1	PROX1	NM_002763	+	0	-2947	1	5629	prospero homeobox 1

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC112	11	13984886	13984972	1%	3%	14%	25%	6%	11%	31%	3.40	16.30	29.63	7.65	12.51	36.31
LC113	5	134879362	134879483	0%	1%	5%	13%	35%	5%	17%	3.15	10.56	29.38	80.54	10.94	39.68
LC114	4	8859990	8860023	1%	3%	13%	31%	21%	15%	34%	2.85	12.04	28.82	19.00	14.14	31.74
LC115	14	52735425	52735485	1%	3%	15%	29%	18%	15%	63%	3.09	15.29	28.53	17.82	14.71	61.82
LC116	18	12254306	12254366	0%	1%	3%	10%	1%	2%	5%	2.01	9.00	28.44	3.39	4.24	14.73
LC117	6	2903614	2903705	0%	1%	2%	13%	21%	2%	1%	1.58	3.90	28.42	45.90	4.89	1.60
LC118	8	72755971	72756053	1%	1%	17%	25%	6%	2%	23%	1.61	19.29	28.37	7.16	1.82	25.68
LC119	8	23564059	23564136	1%	2%	12%	23%	30%	12%	50%	2.76	14.60	27.91	36.65	14.91	61.08
LC120	19	30017795	30017896	1%	1%	13%	19%	10%	3%	14%	1.80	18.06	27.33	13.50	4.20	20.21
LC121	12	25056183	25056246	1%	5%	19%	37%	54%	29%	64%	4.03	13.60	27.03	39.72	21.36	47.09
LC122	12	50297879	50297912	1%	2%	17%	20%	4%	7%	19%	2.26	22.36	27.00	5.77	9.22	24.93
LC123	14	52735223	52735373	1%	2%	12%	22%	14%	7%	62%	2.15	14.58	26.97	17.16	8.92	74.66
LC124	2	25439185	25439264	0%	1%	10%	11%	31%	3%	7%	2.31	25.89	26.41	77.49	6.89	18.37
LC125	1	32237695	32237880	1%	3%	22%	25%	44%	10%	14%	2.78	22.43	25.97	45.37	10.13	14.59
LC126	5	32713586	32713669	2%	5%	25%	42%	17%	11%	33%	3.07	15.16	25.58	10.49	6.68	20.46
LC127	8	145105570	145105675	1%	3%	30%	26%	9%	29%	23%	3.15	29.77	25.41	9.23	28.54	22.59
LC128	12	52401041	52401138	1%	1%	12%	16%	1%	2%	12%	1.36	19.11	25.37	2.04	2.89	19.80
LC129	7	27195748	27195829	1%	5%	25%	36%	38%	24%	69%	3.68	17.21	25.20	26.39	16.93	48.45
LC130	2	99439270	99439356	1%	3%	24%	31%	81%	32%	56%	2.54	19.21	25.16	66.32	26.30	45.23

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC112	11	SPON1	NM_006108	+	0	973	1	10418	spondin 1, extracellular matrix protein
LC113	5	MAX.chr5.134879362-134879483	-	-	0	-	0	-	-
LC114	4	MAX.chr4.8859990-8860023	-	-	0	-	1	-	-
LC115	14	PTGDR	NM_000953	+	0	995	1	5729	prostaglandin D2 receptor (DP)
LC116	18	CIDEA	NM_001279;NR_036468	+	0	-11;-53	1	1149	cell death-inducing DFFA-like effector a
LC117	6	SERPINB9	NM_004155	-	0	-69	1	5272	serpin peptidase inhibitor, clade B (ovalbumin), member 9
LC118	8	LOC100132891	NR_033651;NR_033652	+	1	-379;614	1	100132891	hypothetical LOC100132891
LC119	8	NKX2-6	NM_001136271	-	0	-137	1	137814	NK2 transcription factor related, locus 6 (Drosophila)
LC120	19	VSTM2B	NM_001146339	+	0	305	1	342865	V-set and transmembrane domain containing 2B
LC121	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46210;46210;-861;46210;-174	1	586	branched chain amino-acid transaminase 1, cytosolic
LC122	12	FAIM2	NM_012306	-	0	-159	1	23017	Fas apoptotic inhibitory molecule 2
LC123	14	PTGDR	NM_000953	+	1	793	1	5729	prostaglandin D2 receptor (DP)
LC124	2	MAX.chr2.25439185-25439264	-	-	0	-	1	-	-
LC125	1	MAX.chr1.32237695-32237880	-	-	0	-	0	-	-
LC126	5	NPR3	NM_000908	+	0	1922	1	4883	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
LC127	8	MAX.chr8.145105570-145105675	-	-	0	-	1	-	-
LC128	12	GRASP	NM_181711	+	1	294	1	160622	GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein
LC129	7	HOXA7	NM_006896	-	0	548	1	3204	homeobox A7
LC130	2	C2orf55	NM_207362	-	1	113414	1	343990	chromosome 2 open reading frame 55

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC131	10	124910562	124910715	1%	3%	7%	31%	26%	11%	16%	2.57	5.42	24.87	20.86	8.77	13.03
LC132	7	35293717	35293754	2%	6%	21%	44%	37%	17%	55%	3.29	11.74	24.80	21.20	9.72	31.02
LC133	1	161275580	161275649	1%	1%	6%	20%	1%	12%	2%	1.19	7.61	24.72	1.32	14.12	2.90
LC134	17	44896701	44896855	0%	1%	5%	11%	5%	6%	2%	1.54	10.77	24.70	10.80	12.84	3.79
LC135	6	108440646	108440760	1%	3%	23%	30%	56%	23%	29%	2.73	19.07	24.47	45.83	18.68	23.23
LC136	6	2903051	2903104	1%	1%	2%	16%	9%	3%	0%	1.13	2.43	24.28	13.65	4.40	0.45
LC137	12	57618791	57618831	2%	4%	21%	37%	78%	30%	61%	2.91	13.94	23.92	50.79	19.55	39.93
LC138	21	36042030	36042110	1%	2%	4%	22%	41%	13%	55%	1.88	3.80	23.37	43.13	13.73	57.60
LC139	2	233352635	233352699	1%	1%	8%	18%	3%	3%	18%	1.40	9.63	22.92	3.72	3.82	21.94
LC140	14	52536106	52536240	1%	2%	15%	21%	17%	9%	42%	2.36	16.40	22.80	18.91	10.21	46.04
LC141	10	105036730	105036777	1%	2%	17%	25%	14%	6%	53%	2.01	15.78	22.74	12.57	5.11	48.13
LC142	1	110627121	110627221	1%	3%	7%	25%	39%	7%	33%	2.37	6.55	22.73	36.30	6.54	30.81
LC143	8	143592229	143592285	1%	1%	8%	12%	5%	2%	16%	2.49	15.63	22.64	9.72	4.31	29.27
LC144	7	158938047	158938133	1%	4%	9%	30%	14%	7%	29%	3.07	6.84	22.63	10.90	5.54	22.04
LC145	17	43339264	43339345	1%	1%	4%	11%	41%	12%	11%	1.45	8.50	22.19	82.48	23.48	21.17
LC146	10	119312919	119312997	1%	3%	15%	26%	28%	5%	11%	2.57	12.66	22.08	23.61	4.33	9.25
LC147	17	77179798	77179841	1%	1%	12%	22%	3%	4%	26%	1.14	11.60	21.93	3.21	3.68	26.04
LC148	4	155412291	155412375	1%	1%	5%	12%	6%	2%	10%	2.01	10.12	21.79	11.77	3.65	19.36
LC149	8	70947017	70947084	1%	2%	19%	31%	46%	19%	38%	1.50	13.80	21.67	32.73	13.18	26.96
LC150	15	65116474	65116558	1%	1%	9%	15%	9%	18%	1%	1.58	12.74	21.62	13.62	26.30	1.26
LC151	14	52535777	52535870	1%	2%	6%	14%	6%	4%	21%	2.36	8.67	21.61	8.65	6.18	32.65
LC152	12	103352291	103352323	1%	1%	3%	26%	3%	7%	31%	1.13	2.73	21.57	2.19	5.76	25.28

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC131	10	BUB3	NM_001007793;NM_004725	+	0	-3197;-3197	1	9184	budding uninhibited by benzimidazoles 3 homolog (yeast)
LC132	7	TBX20	NM_001166220;NM_001077653	-	0	-6;-6	1	57057	T-box 20
LC133	1	MPZ	NM_000530	-	1	4182	0	4359	myelin protein zero
LC134	17	WNT3	NM_030753	-	0	-619	1	7473	wingless-type MMTV integration site family, member 3
LC135	6	MAX.chr6.108440646-108440760	-	-	0	-	1	-	-
LC136	6	SERPINB9	NM_004155	-	0	494	0	5272	serpin peptidase inhibitor, clade B (ovalbumin), member 9
LC137	12	NXPH4	NM_007224	+	1	8214	1	11247	neurexophilin 4
LC138	21	CLIC6	NM_053277	+	1	343	1	54102	chloride intracellular channel 6
LC139	2	ECEL1	NM_004826	-	0	-103	1	9427	endothelin converting enzyme-like 1
LC140	14	NID2	NM_007361	-	0	-160	1	22795	nidogen 2 (osteonidogen)
LC141	10	INA	NM_032727	+	0	-189	1	9118	internexin neuronal intermediate filament protein, alpha
LC142	1	MAX.chr1.110627121-110627221	-	-	0	-	1	-	-
LC143	8	BAI1	NM_001702	+	0	46853	1	575	brain-specific angiogenesis inhibitor 1
LC144	7	VIPR2	NM_003382	-	0	-398	1	7434	vasoactive intestinal peptide receptor 2
LC145	17	C17orf46	NM_152343	-	0	215	1	124783	chromosome 17 open reading frame 46
LC146	10	MAX.chr10.119312919-119312997	-	-	0	-	1	-	-
LC147	17	RBFOX3	NM_001082575	-	0	298765	1	146713	RNA binding protein, fox-1 homolog (C. elegans) 3
LC148	4	DCHS2	NM_001142552;NM_001142553	-	1	586;639	1	54798	dachsous 2 (Drosophila)
LC149	8	MAX.chr8.70947017-70947084	-	-	0	-	1	-	-
LC150	15	PIF1	NM_025049	-	1	1364	1	80119	PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae)
LC151	14	NID2	NM_007361	-	1	169	1	22795	nidogen 2 (osteonidogen)
LC152	12	ASCL1	NM_004316	+	1	840	1	429	achaete-scute complex homolog 1 (Drosophila)

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
LC153	9	114075	114141	1%	1%	7%	14%	6%	3%	10%	1.46	11.00	21.51	9.66	4.36	14.91
LC154	2	26407567	26407639	1%	1%	5%	12%	62%	27%	16%	1.73	9.78	21.33	112.60	49.01	28.67
LC155	17	80329497	80329526	2%	4%	15%	35%	15%	7%	18%	2.19	9.12	21.26	9.30	4.01	11.06
LC156	2	946378	946438	1%	1%	10%	13%	4%	3%	20%	2.16	16.72	21.25	7.07	5.17	32.61
LC157	2	87088958	87088997	2%	4%	23%	42%	10%	8%	34%	2.06	11.42	21.20	4.83	4.08	16.97
LC158	1	78511892	78512047	1%	2%	5%	21%	12%	10%	16%	2.00	4.64	21.05	12.33	10.20	15.52
LC159	17	46832435	46832494	2%	4%	18%	51%	64%	10%	45%	1.74	7.35	20.82	26.15	4.19	18.31
LC160	5	1876308	1876340	2%	3%	21%	42%	5%	24%	30%	1.64	10.14	20.71	2.63	11.89	14.89
LC161	4	8859049	8859184	1%	1%	6%	14%	12%	6%	23%	2.12	8.57	20.70	18.12	9.42	34.78
LC162	1	8277482	8277571	1%	2%	10%	22%	48%	31%	59%	1.59	9.26	20.67	45.68	29.57	56.50
LC163	5	10563569	10563607	1%	2%	6%	16%	9%	2%	23%	2.00	7.48	20.66	12.20	2.30	30.00
LC164	2	74726082	74726257	1%	1%	5%	19%	13%	7%	22%	1.42	5.07	20.56	13.46	7.60	23.48
LC165	9	129377645	129377714	1%	1%	2%	12%	2%	7%	4%	1.91	3.68	20.35	3.27	11.71	7.54
LC166	12	106979840	106979932	2%	3%	13%	32%	4%	11%	39%	1.93	7.96	20.27	2.45	7.05	24.97
LC167	3	170137371	170137439	2%	5%	15%	34%	49%	33%	62%	2.78	9.08	20.26	29.02	19.46	36.27
LC168	2	74726270	74726331	1%	1%	6%	24%	22%	9%	22%	1.27	5.44	20.19	19.04	7.96	18.84
LC169	7	50343331	50343395	1%	2%	6%	20%	11%	3%	16%	2.36	5.94	20.12	11.47	2.74	15.82
LC170	8	687683	687729	2%	4%	29%	46%	31%	24%	58%	1.75	12.72	20.04	13.43	10.27	25.10

FIG. 3 (cont'd)

Large cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
LC153	9	MAX.chr9.114075-114141	-	-	0	-	1	-	-
LC154	2	FAM59B	NM_001191033;NM_001168241	+	1	3983;11608	1	150946	family with sequence similarity 59, member B
LC155	17	UTS2R	NM_018949	+	0	-2703	1	2837	urotensin 2 receptor
LC156	2	SNTG2	NM_018968	+	0	-175	1	54221	syntrophin, gamma 2
LC157	2	CD8B	NM_004931;NM_172102;NM_001178100;NM_172213;NM_172101	-	1	89;89;89;89;89	1	926	CD8b molecule
LC158	1	GIPC2	NM_017655	+	1	304	1	54810	GIPC PDZ domain containing family, member 2
LC159	17	MAX.chr17.46832435-46832494	-	-	0	-	1	-	-
LC160	5	MAX.chr5.1876308-1876340	-	-	0	-	1	-	-
LC161	4	MAX.chr4.8859049-8859184	-	-	0	-	1	-	-
LC162	1	MAX.chr1.8277482-8277571	-	-	0	-	1	-	-
LC163	5	ANKRD33B	NM_001164440	+	0	-865	1	651746	ankyrin repeat domain 33B
LC164	2	LBX2	NM_001009812	-	0	4361	1	85474	ladybird homeobox 2
LC165	9	LMX1B	NM_002316;NM_001174146;NM_001174147	+	0	924;924;924	1	4010	LIM homeobox transcription factor 1, beta
LC166	12	RFX4	NM_213594	+	0	2808	1	5992	regulatory factor X, 4 (influences HLA class II expression)
LC167	3	CLDN11	NM_001185056;NM_005602	+	0	-1656;719	1	5010	claudin 11
LC168	2	LBX2	NM_001009812	-	0	4173	1	85474	ladybird homeobox 2
LC169	7	IKZF1	NM_006060	+	0	-1046	1	10320	IKAROS family zinc finger 1 (Ikaros)
LC170	8	MAX.chr8.687683-687729	-	-	0	-	1	-	-

FIG. 4

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC1	5	42995102	42995171	0%	4%	8%	21%	43%	32%	59%	37.37	88.00	220.17	459.69	335.17	627.38
SC2	11	14926886	14926955	0%	3%	6%	29%	78%	21%	20%	8.70	18.97	99.05	266.28	70.91	68.97
SC3	11	14926795	14926853	0%	2%	9%	26%	78%	27%	10%	6.70	27.57	84.20	249.90	86.19	33.11
SC4	5	42995477	42995528	0%	5%	20%	35%	60%	39%	62%	18.40	82.13	141.62	245.53	158.96	254.67
SC5	9	124132797	124132861	0%	3%	6%	8%	49%	10%	55%	15.58	27.54	37.71	217.46	45.00	246.29
SC6	17	27467359	27467467	0%	0%	10%	7%	28%	4%	5%	2.69	61.89	42.49	170.64	26.04	31.95
SC7	11	14926995	14927132	0%	4%	6%	16%	66%	14%	13%	10.65	16.01	42.26	168.75	35.65	33.15
SC8	6	149803478	149803586	0%	4%	6%	16%	48%	19%	25%	14.98	19.63	54.98	164.84	66.15	87.14
SC9	19	17403238	17403270	0%	2%	3%	4%	46%	5%	22%	8.35	9.57	14.66	154.96	15.55	74.38
SC10	3	122296709	122296828	0%	3%	17%	23%	65%	25%	61%	5.87	39.97	54.78	151.39	57.49	143.28
SC11	10	22541891	22541996	0%	2%	13%	22%	49%	18%	24%	5.71	36.56	63.36	141.74	52.16	69.90
SC12	8	72754556	72754648	1%	4%	18%	23%	65%	21%	50%	8.04	33.54	42.57	119.67	39.04	91.67
SC13	7	100075307	100075425	0%	2%	21%	18%	54%	15%	16%	3.87	45.55	39.16	116.74	32.72	34.94
SC14	8	144328573	144328649	0%	2%	10%	13%	32%	7%	22%	5.38	32.52	41.01	101.81	21.45	68.46
SC15	9	96721507	96721564	1%	3%	11%	22%	77%	19%	28%	2.86	11.67	24.03	83.04	20.71	29.71
SC16	10	22624410	22624553	1%	2%	7%	30%	63%	11%	58%	2.80	9.42	38.62	82.32	14.11	75.75
SC17	8	70981960	70982028	1%	4%	26%	36%	65%	28%	58%	5.02	30.29	41.91	74.59	32.63	66.72
SC18	3	138658597	138658705	1%	4%	15%	29%	50%	25%	58%	6.02	21.20	39.97	68.59	34.74	79.20
SC19	2	99439270	99439356	1%	3%	24%	31%	81%	32%	56%	2.54	19.21	25.16	66.32	26.30	45.23
SC20	14	33402226	33402304	1%	4%	24%	21%	71%	15%	49%	3.48	22.10	19.50	64.55	13.58	45.05
SC21	1	223936705	223936773	1%	4%	15%	30%	65%	24%	31%	4.19	14.48	28.80	62.06	22.68	29.92
SC22	7	27205013	27205081	1%	7%	29%	50%	83%	42%	48%	5.20	20.79	36.52	60.30	30.77	35.13
SC23	17	48042562	48042606	1%	3%	24%	47%	56%	25%	44%	3.67	25.97	50.24	59.12	26.78	46.42
SC24	11	31820365	31820418	1%	4%	19%	39%	74%	21%	59%	3.41	14.93	30.02	57.24	16.07	45.33
SC25	2	71116233	71116269	1%	4%	14%	24%	62%	15%	51%	4.02	12.56	21.37	55.76	13.59	46.07
SC26	2	239140226	239140351	1%	2%	7%	16%	42%	5%	15%	2.14	8.36	19.00	51.66	6.30	17.68
SC27	18	55095158	55095201	1%	5%	11%	31%	64%	13%	42%	3.65	9.00	23.92	50.49	10.38	32.83
SC28	15	89914730	89914776	1%	3%	4%	10%	59%	18%	16%	2.44	3.73	8.26	49.97	15.08	13.65
SC29	2	74726554	74726617	1%	4%	38%	47%	68%	31%	49%	3.10	27.46	34.21	49.28	22.18	35.41

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC1	5	MAX.chr5.42995102-42995171	-	-	0	-	0	-	-
SC2	11	MAX.chr11.14926886-14926955	-	-	0	-	1	-	-
SC3	11	MAX.chr11.14926795-14926853	-	-	0	-	1	-	-
SC4	5	MAX.chr5.42995477-42995528	-	-	0	-	0	-	-
SC5	9	STOM	NM_198194;NM_004099	-	0	-252;-252	1	2040	stomatin
SC6	17	MYO18A	NM_078471;NM_203318	-	0	40048;40048	0	399687	myosin XVIIIA
SC7	11	MAX.chr11.14926995-14927132	-	-	0	-	1	-	-
SC8	6	ZC3H12D	NM_207360	-	0	2670	0	340152	zinc finger CCCH-type containing 12D
SC9	19	ABHD8	NM_024527	-	1	11044	1	79575	abhydrolase domain containing 8
SC10	3	PARP15	NM_001113523	+	0	261	1	165631	poly (ADP-ribose) polymerase family, member 15
SC11	10	MAX.chr10.22541891-22541996	-	-	0	-	1	-	-
SC12	8	LOC100132891	NR_033651;NR_033652	+	1	-1794;-801	1	100132891	hypothetical LOC100132891
SC13	7	TSC22D4	NM_030935	-	1	1595	1	81628	TSC22 domain family, member 4
SC14	8	ZFP41	NM_173832	+	0	-535	1	286128	zinc finger protein 41 homolog (mouse)
SC15	9	BARX1	NM_021570	-	0	-3899	1	56033	BARX homeobox 1
SC16	10	MAX.chr10.22624410-22624553	-	-	0	-	1	-	-
SC17	8	PRDM14	NM_024504	-	1	1602	1	63978	PR domain containing 14
SC18	3	MAX.chr3.138658597-138658705	-	-	0	-	1	-	-
SC19	2	C2orf55	NM_207362	-	1	113414	1	343990	chromosome 2 open reading frame 55
SC20	14	MAX.chr14.33402226-33402304	-	-	0	-	1	-	-
SC21	1	CAPN2	NM_001146068;NM_001748	+	0	47411;36587	1	824	calpain 2, (m/II) large subunit
SC22	7	HOXA9	NM_152739	-	1	136	1	3205	homeobox A9
SC23	17	DLX4	NM_138281	+	0	-3999	1	1748	distal-less homeobox 4
SC24	11	PAX6	NM_000280;NM_001604;NM_001127612	-	0	12514;12514;19144	1	5080	paired box 6
SC25	2	MAX.chr2.71116233-71116269	-	-	0	-	1	-	-
SC26	2	LOC151174	NR_026926;NR_026925	-	1	92;92	1	151174	hypothetical LOC151174
SC27	18	MAX.chr18.55095158-55095201	-	-	0	-	1	-	-
SC28	15	MAX.chr15.89914730-89914776	-	-	0	-	1	-	-
SC29	2	LBX2	NM_001009812	-	0	3889	1	85474	ladybird homeobox 2

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC30	12	133481464	133481521	1%	5%	25%	28%	54%	26%	49%	4.21	22.69	24.67	48.26	23.58	43.20
SC31	10	22624260	22624375	1%	2%	6%	21%	53%	13%	56%	1.91	5.10	18.41	47.17	11.24	50.06
SC32	20	61560462	61560535	2%	3%	9%	19%	72%	30%	26%	1.81	5.48	11.30	42.16	17.75	15.47
SC33	1	151811410	151811523	2%	8%	20%	39%	68%	16%	34%	4.65	12.47	24.13	42.10	9.64	20.83
SC34	5	1295444	1295496	2%	3%	16%	28%	76%	15%	58%	1.93	8.89	15.80	42.04	8.35	32.46
SC35	7	8482598	8482670	2%	4%	19%	34%	80%	14%	54%	2.23	9.82	17.57	41.49	7.09	27.92
SC36	9	96722680	96722762	2%	4%	13%	18%	81%	18%	54%	1.81	6.40	9.21	40.62	9.17	27.11
SC37	19	13617166	13617235	2%	3%	16%	24%	79%	7%	42%	1.52	7.95	12.14	40.60	3.55	21.30
SC38	11	31825851	31825955	1%	5%	12%	28%	56%	18%	49%	3.29	8.47	20.02	40.60	12.92	35.78
SC39	1	47696594	47696674	1%	4%	13%	16%	46%	9%	51%	3.76	10.92	13.85	39.04	7.83	43.54
SC40	5	1295519	1295587	2%	4%	17%	28%	72%	11%	60%	2.15	9.27	15.05	38.74	6.15	32.15
SC41	19	16394457	16394575	1%	2%	18%	14%	52%	21%	23%	1.72	13.16	10.15	38.09	15.47	17.08
SC42	14	101033663	101033775	1%	2%	12%	8%	32%	4%	15%	1.82	13.80	9.61	37.74	5.12	17.01
SC43	2	71116047	71116131	2%	5%	20%	37%	73%	21%	66%	2.54	10.30	19.16	37.51	11.04	34.09
SC44	3	157821297	157821378	2%	5%	15%	35%	77%	33%	48%	2.18	7.42	16.95	37.26	15.88	23.32
SC45	20	39597822	39597893	1%	5%	8%	7%	50%	8%	38%	3.36	5.53	5.14	36.66	5.59	27.57
SC46	8	23564059	23564136	1%	2%	12%	23%	30%	12%	50%	2.76	14.60	27.91	36.65	14.91	61.08
SC47	20	25061836	25061911	1%	4%	19%	19%	54%	8%	55%	2.39	12.78	12.56	36.50	5.35	36.91
SC48	2	171678927	171678966	2%	5%	23%	43%	83%	36%	56%	2.35	10.01	18.83	36.28	15.71	24.53
SC49	1	165323561	165323624	2%	5%	18%	29%	62%	19%	60%	2.83	10.55	16.83	35.93	10.85	34.56
SC50	4	2765684	2765768	2%	5%	13%	20%	73%	13%	44%	2.44	6.44	9.67	35.86	6.17	21.87
SC51	14	38724873	38724946	2%	5%	26%	28%	54%	28%	39%	3.19	17.27	18.37	35.74	18.39	25.84

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC30	12	MAX.chr12.133481464-133481521	-	-	0	-	0	-	-
SC31	10	MAX.chr10.22624260-22624375	-	-	0	-	1	-	-
SC32	20	DIDO1	NM_033081;NM_001193369;NM_022105;NM_080797;NM_001193370;NM_080796	-	0	8842;-2559;8842;8842;-2559;-2559	1	11083	death inducer-obliterator 1
SC33	1	C2CD4D	NM_001136003	-	1	1623	1	100191040	C2 calcium-dependent domain containing 4D
SC34	5	TERT	NM_001193376;NM_198253	-	0	-282;-282	1	7015	telomerase reverse transcriptase
SC35	7	NXPH1	NM_152745	+	0	9014	1	30010	neurexophilin 1
SC36	9	MAX.chr9.96722680-96722762	-	-	0	-	1	-	-
SC37	19	CACNA1A	NM_001127222;NM_023035;NM_001127221;NM_000068;NM_001174080	-	1	108;108;108;108;108	1	773	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit
SC38	11	PAX6	NM_000280;NM_001604;NM_001127612	-	0	7028;7028;13658	1	5080	paired box 6
SC39	1	TAL1	NM_003189	-	0	-1151	1	6886	T-cell acute lymphocytic leukemia 1
SC40	5	TERT	NM_001193376;NM_198253	-	0	-357;-357	1	7015	telomerase reverse transcriptase
SC41	19	MAX.chr19.16394457-16394575	-	-	0	-	1	-	-
SC42	14	BEGAIN	NM_001159531;NM_020836	-	0	744;2468	0	57596	brain-enriched guanylate kinase-associated homolog (rat)
SC43	2	MAX.chr2.71116047-71116131	-	-	0	-	1	-	-
SC44	3	SHOX2	NM_001163678;NM_006884;NM_003030	-	0	2655;2655;2655	1	6474	short stature homeobox 2
SC45	20	MAX.chr20.39597822-39597893	-	-	0	-	0	-	-
SC46	8	NKX2-6	NM_001136271	-	0	-137	1	137814	NK2 transcription factor related, locus 6 (Drosophila)
SC47	20	VSX1	NM_014588;NM_199425	-	0	931;931	1	30813	visual system homeobox 1
SC48	2	GAD1	NM_000817;NM_013445	+	0	5728;5728	1	2571	glutamate decarboxylase 1 (brain, 67kDa)
SC49	1	LMX1A	NM_001174069;NM_177398	-	0	1917;2391	1	4009	LIM homeobox transcription factor 1, alpha
SC50	4	MAX.chr4.2765684-2765768	-	-	0	-	1	-	-
SC51	14	CLEC14A	NM_175060	-	1	701	1	161198	C-type lectin domain family 14, member A

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC52	14	57275051	57275128	2%	5%	23%	23%	69%	33%	62%	2.46	12.11	12.16	35.71	17.08	32.11
SC53	1	119527180	119527255	2%	5%	18%	33%	66%	26%	59%	2.62	9.32	17.22	34.40	13.57	30.76
SC54	17	37321375	37321560	1%	2%	14%	16%	46%	20%	28%	1.69	10.17	11.26	33.55	14.38	20.12
SC55	19	2282589	2282652	2%	4%	17%	18%	70%	8%	34%	1.95	8.09	8.43	33.32	3.73	16.12
SC56	14	57275166	57275267	2%	3%	17%	15%	58%	23%	58%	1.81	9.89	8.63	33.21	12.99	32.92
SC57	8	70947017	70947084	1%	2%	19%	31%	46%	19%	38%	1.50	13.80	21.67	32.73	13.18	26.96
SC58	17	37321636	37321779	2%	4%	25%	28%	56%	24%	35%	2.13	14.57	16.29	32.47	13.78	20.19
SC59	7	156796836	156796900	2%	6%	34%	35%	65%	13%	44%	3.16	16.78	17.33	32.09	6.32	22.05
SC60	13	112708072	112708131	2%	4%	16%	19%	60%	29%	52%	2.28	8.17	9.98	30.61	15.09	26.55
SC61	15	60287478	60287520	3%	3%	7%	24%	75%	11%	56%	1.34	2.93	9.22	29.60	4.15	21.93
SC62	2	176964778	176964812	3%	5%	29%	35%	80%	40%	78%	1.72	10.48	12.64	28.72	14.52	27.93
SC63	17	59529152	59529199	2%	4%	8%	27%	64%	27%	19%	1.93	3.71	12.10	28.70	11.98	8.64
SC64	16	79623678	79623752	2%	4%	14%	9%	60%	12%	31%	1.67	6.46	4.23	28.57	5.92	14.79
SC65	9	37002603	37002649	3%	4%	18%	31%	74%	17%	51%	1.40	6.83	11.99	28.51	6.44	19.72
SC66	17	35299945	35299975	3%	7%	34%	41%	70%	30%	67%	2.75	13.34	15.98	27.54	11.92	26.15
SC67	9	96714376	96714430	3%	5%	22%	26%	84%	23%	35%	1.73	7.31	8.50	27.40	7.43	11.57
SC68	8	23564008	23564050	1%	4%	16%	23%	33%	14%	48%	2.96	12.99	19.51	27.33	11.90	40.25
SC69	2	162280520	162280586	2%	5%	25%	32%	61%	22%	48%	2.23	11.24	14.21	27.13	9.78	21.69
SC70	17	35300794	35300829	2%	6%	17%	32%	62%	19%	36%	2.40	6.98	13.40	26.28	7.88	14.98
SC71	14	60952425	60952483	2%	4%	16%	17%	57%	16%	53%	1.86	7.61	7.86	26.23	7.50	24.42
SC72	7	156814766	156814822	2%	2%	10%	17%	47%	7%	25%	1.21	5.45	9.24	25.65	3.68	13.51
SC73	17	46711207	46711240	2%	7%	27%	33%	61%	22%	46%	2.92	11.03	13.61	24.97	8.80	18.91
SC74	11	74178467	74178613	2%	3%	4%	8%	50%	5%	10%	1.26	2.15	4.04	24.88	2.51	5.18
SC75	5	42993534	42993659	2%	5%	10%	21%	53%	18%	49%	2.27	4.60	9.88	24.87	8.31	22.95
SC76	5	72595672	72595716	3%	6%	21%	29%	73%	17%	66%	2.06	7.13	9.82	24.53	5.88	22.18
SC77	19	13617366	13617505	3%	4%	14%	12%	70%	6%	34%	1.32	4.91	4.33	24.48	2.22	11.85

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC52	14	OTX2	NM_172337;NM_021728	-	0	-2706;2133	1	5015	orthodenticle homeobox 2
SC53	1	TBX15	NM_152380	-	0	4999	1	6913	T-box 15
SC54	17	ARL5C	NM_001143968	-	1	1039	0	390790	ADP-ribosylation factor-like 5C
SC55	19	C19orf35	NM_198532	-	0	-408	0	374872	chromosome 19 open reading frame 35
SC56	14	OTX2	NM_172337;NM_021728	-	0	-2821;2018	1	5015	orthodenticle homeobox 2
SC57	8	MAX.chr8.70947017-70947084	-	-	0	-	1	-	-
SC58	17	ARL5C	NM_001143968	-	0	778	1	390790	ADP-ribosylation factor-like 5C
SC59	7	MAX.chr7.156796836-156796900	-	-	0	-	1	-	-
SC60	13	MAX.chr13.112708072-112708131	-	-	0	-	1	-	-
SC61	15	MAX.chr15.60287478-60287520	-	-	0	-	1	-	-
SC62	2	HOXD12	NM_021193	+	1	249	1	3238	homeobox D12
SC63	17	TBX4	NM_018488	+	0	-4654	1	9496	T-box 4
SC64	16	MAX.chr16.79623678-79623752	-	-	0	-	1	-	-
SC65	9	PAX5	NM_016734	-	0	31873	1	5079	paired box 5
SC66	17	LHX1	NM_005568	+	0	5174	1	3975	LIM homeobox 1
SC67	9	BARX1	NM_021570	-	1	3232	1	56033	BARX homeobox 1
SC68	8	NKX2-6	NM_001136271	-	0	-86	1	137814	NK2 transcription factor related, locus 6 (Drosophila)
SC69	2	TBR1	NM_006593	+	1	7901	1	10716	T-box, brain, 1
SC70	17	LHX1	NM_005568	+	1	6023	1	3975	LIM homeobox 1
SC71	14	C14orf39	NM_174978	-	0	339	1	317761	chromosome 14 open reading frame 39
SC72	7	MAX.chr7.156814766-156814822	-	-	0	-	0	-	-
SC73	17	MIR196A1	NR_029582	-	0	-1286	1	406972	microRNA 196a-1
SC74	11	KCNE3	NM_005472	-	1	133	1	10008	potassium voltage-gated channel, Isk-related family, member 3
SC75	5	MAX.chr5.42993534-42993659	-	-	0	-	0	-	-
SC76	5	MAX.chr5.72595672-72595716	-	-	0	-	1	-	-
SC77	19	CACNA1A	NM_001127222;NM_023035;NM_001127221;NM_000068;NM_001174080	-	0	-92;-92;-92;-92;-92	0	773	calcium channel, voltage-dependent, P/Q type, alpha 1A subunit

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC78	11	74178305	74178408	2%	2%	4%	9%	49%	5%	13%	1.05	2.00	4.30	23.91	2.45	6.46
SC79	1	50885167	50885250	3%	5%	19%	22%	79%	31%	60%	1.35	5.67	6.59	23.78	9.13	18.04
SC80	3	138659030	138659082	2%	8%	27%	27%	59%	25%	36%	3.18	10.80	10.88	23.77	10.05	14.63
SC81	6	45631289	45631363	3%	6%	14%	34%	59%	20%	74%	2.29	5.52	13.60	23.49	8.10	29.57
SC82	6	10421453	10421550	3%	6%	14%	28%	68%	23%	50%	2.12	4.84	9.60	23.21	7.79	17.17
SC83	11	20627327	20627367	3%	5%	15%	16%	74%	11%	43%	1.49	4.59	5.07	23.06	3.31	13.58
SC84	9	37030436	37030506	3%	4%	9%	11%	71%	7%	24%	1.33	2.87	3.44	22.93	2.40	7.84
SC85	3	138658429	138658507	2%	5%	15%	35%	43%	23%	52%	2.49	8.08	18.45	22.68	12.15	27.67
SC86	7	8482447	8482526	3%	6%	22%	35%	70%	17%	55%	1.84	7.01	11.06	22.36	5.51	17.36
SC87	7	157478020	157478086	4%	6%	19%	28%	80%	17%	63%	1.53	5.18	7.78	22.02	4.63	17.28
SC88	1	149672622	149672714	2%	4%	12%	20%	50%	19%	33%	1.78	5.42	8.91	21.77	8.39	14.44
SC89	9	96588741	96588774	4%	5%	23%	24%	76%	18%	60%	1.40	6.54	6.62	21.42	5.00	16.79
SC90	6	106429477	106429533	3%	6%	14%	25%	55%	17%	37%	2.21	5.42	9.91	21.29	6.62	14.36
SC91	9	96714225	96714363	3%	4%	14%	17%	58%	15%	20%	1.50	5.03	6.36	21.29	5.43	7.33
SC92	17	35300086	35300164	3%	6%	23%	44%	67%	23%	66%	1.90	7.45	13.87	21.26	7.40	20.98
SC93	15	89914568	89914681	3%	4%	9%	15%	70%	26%	25%	1.31	2.63	4.57	20.96	7.72	7.48
SC94	11	31827697	31827758	3%	4%	10%	39%	68%	21%	16%	1.29	2.95	11.78	20.63	6.35	4.88
SC95	2	239140132	239140170	3%	5%	19%	21%	57%	11%	26%	1.73	6.70	7.55	20.48	4.03	9.37
SC96	2	177001697	177001736	3%	5%	21%	34%	60%	29%	67%	1.62	6.93	11.53	20.37	9.82	22.55
SC97	2	175193478	175193572	3%	5%	7%	18%	59%	25%	32%	1.64	2.40	6.03	20.11	8.41	10.89
SC98	2	176931886	176931980	4%	6%	24%	32%	75%	36%	66%	1.63	6.52	8.48	20.07	9.48	17.58

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC78	11	KCNE3	NM_005472	-	0	295	1	10008	potassium voltage-gated channel, Isk-related family, member 3
SC79	1	DMRTA2	NM_032110	-	1	3974	1	63950	DMRT-like family A2
SC80	3	MAX.chr3.138659030-138659082	-	-	0	-	1	-	-
SC81	6	MAX.chr6.45631289-45631363	-	-	0	-	1	-	-
SC82	6	TFAP2A	NM_001042425	-	0	-1656	1	7020	transcription factor AP-2 alpha (activating enhancer binding protein 2 alpha)
SC83	11	SLC6A5	NM_004211	+	0	6382	0	9152	solute carrier family 6 (neurotransmitter transporter, glycine), member 5
SC84	9	PAX5	NM_016734	-	0	4040	0	5079	paired box 5
SC85	3	MAX.chr3.138658429-138658507	-	-	0	-	1	-	-
SC86	7	NXPH1	NM_152745	+	0	8863	1	30010	neurexophilin 1
SC87	7	PTPRN2	NM_130842;NM_002847;NM_130843	-	0	902462;902462;902462	1	5799	protein tyrosine phosphatase, receptor type, N polypeptide 2
SC88	1	MAX.chr1.149672622-149672714	-	-	0	-	1	-	-
SC89	9	MAX.chr9.96588741-96588774	-	-	0	-	1	-	-
SC90	6	MAX.chr6.106429477-106429533	-	-	0	-	1	-	-
SC91	9	BARX1	NM_021570	-	1	3383	1	56033	BARX homeobox 1
SC92	17	LHX1	NM_005568	+	1	5315	1	3975	LIM homeobox 1
SC93	15	MAX.chr15.89914568-89914681	-	-	0	-	1	-	-
SC94	11	PAX6	NM_000280;NM_001604;NM_001127612	-	0	5182;5182;11812	1	5080	paired box 6
SC95	2	LOC151174	NR_026926;NR_026925	-	1	186;186	1	151174	hypothetical LOC151174
SC96	2	MAX.chr2.177001697-177001736	-	-	0	-	1	-	-
SC97	2	MAX.chr2.175193478-175193572	-	-	0	-	1	-	-
SC98	2	MAX.chr2.176931886-176931980	-	-	0	-	1	-	-

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC99	14	60952634	60952756	3%	5%	14%	20%	57%	15%	59%	1.90	4.95	7.09	20.00	5.23	20.49
SC100	11	14926627	14926716	1%	1%	5%	16%	62%	16%	11%	1.78	10.57	30.66	119.53	31.77	21.41
SC101	2	66666637	66666685	1%	7%	32%	35%	66%	28%	67%	5.39	24.98	27.27	51.35	22.00	51.95
SC102	1	6269157	6269209	1%	7%	26%	22%	61%	23%	61%	5.21	19.42	15.89	45.08	17.31	44.99
SC103	17	72353079	72353146	2%	3%	20%	32%	84%	10%	74%	1.79	10.73	17.31	44.61	5.54	39.28
SC104	1	39980533	39980614	2%	6%	18%	13%	68%	13%	77%	3.63	10.23	7.34	39.03	7.64	44.50
SC105	10	8097914	8097973	1%	5%	19%	31%	54%	21%	52%	3.35	12.62	20.86	36.48	14.17	35.54
SC106	17	27038627	27038718	1%	1%	6%	4%	23%	6%	12%	1.91	8.68	5.96	35.81	9.42	17.97
SC107	9	972186	972250	3%	5%	10%	31%	63%	14%	39%	1.89	3.69	11.26	22.88	4.95	14.22
SC108	8	76316557	76316616	2%	4%	16%	17%	42%	19%	48%	1.88	8.48	8.63	22.16	9.85	25.03
SC109	4	155663905	155663938	2%	4%	21%	28%	32%	13%	31%	2.76	13.76	18.57	21.03	8.54	20.27
SC110	17	27940477	27940568	0%	1%	5%	9%	59%	22%	25%	3.44	21.98	34.20	238.86	87.69	99.96
SC111	1	2165761	2165877	1%	2%	14%	17%	58%	9%	21%	3.72	23.15	27.86	92.49	13.90	33.78
SC112	10	94822422	94822464	1%	8%	11%	30%	63%	19%	54%	5.15	7.55	20.17	42.86	13.14	37.12
SC113	19	31842678	31842822	1%	2%	10%	17%	27%	7%	9%	2.58	10.59	19.10	30.03	7.96	9.88
SC114	12	54812201	54812346	1%	3%	14%	15%	28%	8%	13%	2.51	13.59	13.91	26.63	7.20	12.05
SC115	1	110626702	110626798	0%	2%	12%	17%	59%	11%	48%	5.10	25.49	36.85	129.67	24.94	105.67
SC116	14	65007294	65007341	0%	0%	0%	3%	31%	3%	45%	0.68	0.87	8.06	84.42	7.09	121.70
SC117	12	104609954	104610035	1%	3%	2%	19%	68%	10%	23%	5.01	3.26	30.04	104.45	14.99	35.53
SC118	2	97193509	97193639	1%	1%	14%	7%	16%	13%	16%	2.04	26.57	14.00	30.21	23.54	29.84
SC119	22	19754481	19754550	0%	6%	18%	24%	48%	33%	46%	16.82	56.38	72.02	146.30	101.74	140.35
SC120	7	121950506	121950608	1%	1%	8%	11%	44%	15%	35%	0.98	5.54	7.95	30.55	10.22	24.53
SC121	19	17791129	17791220	0%	0%	2%	1%	53%	1%	15%	1.35	4.63	4.30	158.54	2.85	45.50
SC122	22	28198280	28198349	0%	1%	4%	3%	22%	3%	16%	2.42	11.11	8.66	64.31	7.42	45.69
SC123	2	97193166	97193253	0%	1%	13%	8%	18%	12%	21%	1.71	42.20	25.79	55.43	37.48	67.24

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC99	14	C14orf39	NM_174978	-	0	130	1	317761	chromosome 14 open reading frame 39
SC100	11	MAX.chr11.14926627-14926716	-	-	0	-	1	-	-
SC101	2	MEIS1	NM_002398	+	0	4106	0	4211	Meis homeobox 1
SC102	1	RNF207	NM_207396	+	0	2969	1	388591	ring finger protein 207
SC103	17	BTBD17	NM_001080466	-	1	4879	1	388419	BTB (POZ) domain containing 17
SC104	1	BMP8A	NM_181809	+	0	23216	1	353500	bone morphogenetic protein 8a
SC105	10	FLJ45983	NR_024255;NR_024256	-	0	-2467;-2467	1	399717	hypothetical LOC399717
SC106	17	PROCA1	NM_152465	-	1	245	1	147011	protein interacting with cyclin A1
SC107	9	DMRT3	NM_021240	+	0	-4777	1	58524	doublesex and mab-3 related transcription factor 3
SC108	8	MAX.chr8.76316557-76316616	-	-	0	-	0	-	-
SC109	4	LRAT	NM_004744	+	0	-1257	1	9227	lecithin retinol acyltransferase (phosphatidylcholine--retinol O-acyltransferase)
SC110	17	ANKRD13B	NM_152345	+	1	19951	1	124930	ankyrin repeat domain 13B
SC111	1	SKI	NM_003036	+	0	5628	0	6497	v-ski sarcoma viral oncogene homolog (avian)
SC112	10	CYP26C1	NM_183374	+	0	1402	1	340665	cytochrome P450, family 26, subfamily C, polypeptide 1
SC113	19	TSHZ3	NM_020856	-	0	-2488	1	57616	teashirt zinc finger homeobox 3
SC114	12	ITGA5	NM_002205	-	0	849	1	3678	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
SC115	1	MAX.chr1.110626702-110626798	-	-	0	-	1	-	-
SC116	14	HSPA2	NM_021979	+	1	109	1	3306	heat shock 70kDa protein 2
SC117	12	TXNRD1	NM_001093771	+	0	396	1	7296	thioredoxin reductase 1
SC118	2	MAX.chr2.97193509-97193639	-	-	0	-	0	-	-
SC119	22	TBX1	NM_005992;NM_080647;NM_080646	+	1	10256;10256;10256	1	6899	T-box 1
SC120	7	MAX.chr7.121950506-121950608	-	-	0	-	1	-	-
SC121	19	UNC13A	NM_001080421	-	0	7879	1	23025	unc-13 homolog A (C. elegans)
SC122	22	MN1	NM_002430	-	0	-794	1	4330	meningioma (disrupted in balanced translocation) 1
SC123	2	MAX.chr2.97193166-97193253	-	-	0	-	1	-	-

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC124	6	1620240	1620379	1%	6%	4%	10%	45%	21%	12%	4.88	2.92	8.66	36.89	17.72	10.09
SC125	6	27064644	27064775	1%	3%	11%	11%	38%	6%	23%	2.23	7.75	7.75	25.78	4.29	15.70
SC126	8	145013661	145013775	0%	0%	26%	6%	32%	2%	10%	5.69	361.21	80.01	444.31	30.71	144.38
SC127	14	101033514	101033620	0%	2%	18%	7%	40%	5%	14%	4.29	36.37	14.15	82.74	10.45	29.35
SC128	1	32237893	32237998	1%	2%	19%	29%	52%	10%	27%	2.51	24.19	36.61	65.03	12.62	34.40
SC129	17	58217191	58217358	1%	1%	6%	9%	30%	1%	20%	1.26	5.55	8.49	27.20	1.31	18.28
SC130	17	72350351	72350446	2%	2%	10%	8%	54%	11%	21%	1.36	5.77	4.55	31.81	6.33	12.32
SC131	19	46380017	46380063	1%	2%	7%	13%	43%	8%	20%	2.99	8.59	17.60	57.01	10.67	26.25
SC132	2	118982155	118982248	2%	6%	24%	41%	60%	34%	51%	2.62	11.31	19.18	28.19	15.99	23.95
SC133	2	85361426	85361486	2%	3%	6%	9%	42%	15%	22%	1.26	3.07	4.62	20.61	7.25	10.58
SC134	9	131007357	131007440	1%	2%	7%	10%	20%	5%	11%	1.66	7.04	9.96	20.01	5.36	11.03
SC135	9	35675856	35675991	1%	1%	9%	10%	23%	7%	23%	1.38	11.08	12.62	28.05	8.69	28.17
SC136	8	124173236	124173386	0%	1%	11%	15%	49%	14%	29%	7.50	63.79	86.89	276.90	78.49	166.63
SC137	8	108509567	108509637	1%	1%	14%	3%	62%	7%	58%	1.01	15.24	3.44	67.53	8.05	63.61
SC138	8	72754380	72754425	1%	6%	31%	30%	74%	31%	62%	4.73	24.20	23.17	57.79	24.17	48.85
SC139	9	123631470	123631561	0%	1%	0%	2%	37%	2%	6%	1.90	1.41	6.49	124.61	6.16	20.92
SC140	12	57618791	57618831	2%	4%	21%	37%	78%	30%	61%	2.91	13.94	23.92	50.79	19.55	39.93
SC141	19	17958807	17958893	0%	3%	10%	10%	22%	9%	14%	42.72	161.42	159.54	345.13	136.09	214.13
SC142	2	43451713	43451822	0%	2%	4%	11%	61%	9%	14%	8.52	18.71	44.66	258.11	38.21	56.72
SC143	12	1906517	1906559	0%	1%	0%	6%	27%	1%	7%	7.16	3.35	49.38	217.21	10.98	54.50
SC144	9	21965528	21965573	1%	1%	2%	11%	75%	13%	27%	1.65	2.60	13.05	89.72	16.03	31.66
SC145	10	94822484	94822576	1%	5%	8%	22%	58%	16%	31%	4.97	7.48	20.48	53.91	15.39	29.40
SC146	19	39993535	39993600	1%	2%	8%	10%	76%	9%	43%	1.71	5.62	6.73	53.20	5.98	29.81
SC147	6	163837552	163837584	1%	1%	1%	1%	37%	1%	2%	0.73	0.85	1.06	47.13	0.91	2.76

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC124	6	MAX.chr6.1620240-1620379	-	-	0	-	1	-	-
SC125	6	MAX.chr6.27064644-27064775	-	-	0	-	1	-	-
SC126	8	PLEC	NM_201381;NM_201383;NM_000445;NM_201378;NM_201380;NM_201384;NM_201382;NM_201379	-	1	5244;3031;37252;34036;11383;97;4449;14427	0	5339	plectin
SC127	14	BEGAIN	NM_001159531;NM_020836	-	0	893;2617	0	57596	brain-enriched guanylate kinase-associated homolog (rat)
SC128	1	MAX.chr1.32237893-32237998	-	-	0	-	1	-	-
SC129	17	MAX.chr17.58217191-58217358	-	-	0	-	1	-	-
SC130	17	KIF19	NM_153209	+	1	28001	1	124602	kinesin family member 19
SC131	19	MAX.chr19.46380017-46380063	-	-	0	-	1	-	-
SC132	2	MAX.chr2.118982155-118982248	-	-	0	-	1	-	-
SC133	2	TCF7L1	NM_031283	+	0	844	1	83439	transcription factor 7-like 1 (T-cell specific, HMG-box)
SC134	9	DNM1	NM_001005336;NM_004408	+	0	41695;41695	0	1759	dynamin 1
SC135	9	CA9	NM_001216	+	1	1942	1	768	carbonic anhydrase IX
SC136	8	MAX.chr8.124173236-124173386	-	-	0	-	1	-	-
SC137	8	ANGPT1	NM_001146;NM_001199859	-	1	687;687	0	284	angiopoietin 1
SC138	8	LOC100132891	NR_033651;NR_033652	+	1	-1970;-977	1	100132891	hypothetical LOC100132891
SC139	9	PHF19	NM_015651	-	1	8136	1	26147	PHD finger protein 19
SC140	12	NXPH4	NM_007224	+	1	8214	1	11247	neurexophilin 4
SC141	19	JAK3	NM_000215	-	1	34	1	3718	Janus kinase 3
SC142	2	LOC100129726	NR_027251	+	1	-2636	1	100129726	hypothetical LOC100129726
SC143	12	CACNA2D4	NM_172364	-	0	121353	1	93589	calcium channel, voltage-dependent, alpha 2/delta subunit 4
SC144	9	C9orf53	NR_024274	+	0	-1609	1	51198	chromosome 9 open reading frame 53
SC145	10	CYP26C1	NM_183374	+	1	1464	1	340665	cytochrome P450, family 26, subfamily C, polypeptide 1
SC146	19	DLL3	NM_016941;NM_203486	+	1	3979;3979	1	10683	delta-like 3 (Drosophila)
SC147	6	LOC100526820	NR_037593	-	0	-2570	0	-	-

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC148	6	108440646	108440760	1%	3%	23%	30%	56%	23%	29%	2.73	19.07	24.47	45.83	18.68	23.23
SC149	3	184099224	184099335	1%	4%	18%	10%	59%	26%	24%	2.97	13.39	7.63	43.10	19.14	17.55
SC150	14	92980747	92980787	2%	3%	3%	4%	50%	7%	17%	1.85	2.10	2.30	32.86	4.78	10.93
SC151	3	128201932	128202122	1%	7%	21%	18%	60%	22%	40%	4.99	15.77	13.22	44.94	16.26	29.75
SC152	11	518925	518978	0%	1%	1%	1%	28%	3%	7%	3.52	2.42	3.68	135.42	14.75	33.97
SC153	10	102497213	102497267	2%	4%	11%	23%	33%	19%	21%	2.47	6.79	14.51	20.88	12.23	13.18
SC154	20	61560692	61560749	0%	1%	6%	13%	62%	19%	28%	5.65	24.19	56.55	258.74	79.08	115.84
SC155	12	1906566	1906598	0%	2%	1%	7%	32%	1%	8%	8.47	4.68	33.66	163.92	7.51	38.65
SC156	19	15695457	15695579	1%	1%	3%	2%	41%	1%	40%	1.76	5.19	3.78	77.31	2.65	76.95
SC157	1	17215983	17216025	1%	2%	3%	13%	34%	11%	17%	1.88	3.55	15.12	38.96	12.50	19.16
SC158	5	10565409	10565490	1%	2%	24%	22%	50%	7%	31%	1.84	17.95	16.36	37.52	5.45	23.84
SC159	17	72353261	72353424	2%	4%	13%	24%	58%	9%	59%	2.30	7.21	13.50	32.83	5.34	33.27
SC160	5	72732853	72732891	0%	3%	7%	12%	43%	9%	44%	8.51	21.79	35.57	125.15	26.22	126.99
SC161	17	37856733	37856801	2%	1%	2%	1%	67%	2%	17%	0.76	1.07	0.73	41.58	0.98	10.58
SC162	2	26407721	26407876	0%	1%	2%	11%	47%	18%	16%	2.39	8.36	36.01	160.15	60.97	56.07
SC163	19	13950008	13950055	1%	0%	6%	4%	40%	7%	21%	0.28	8.91	6.93	63.59	10.57	33.61
SC164	1	203236695	203236828	0%	0%	1%	2%	11%	3%	2%	0.95	1.76	6.71	35.95	8.17	6.07
SC165	16	70771681	70771770	1%	3%	17%	18%	53%	17%	28%	4.46	24.22	25.13	76.49	23.80	39.51
SC166	12	58021623	58021670	1%	7%	16%	30%	68%	27%	26%	5.02	11.65	22.46	50.71	20.53	19.86
SC167	16	54971076	54971118	2%	5%	7%	19%	62%	27%	27%	2.10	2.81	7.63	25.26	10.87	11.18
SC168	14	38724686	38724772	2%	4%	22%	23%	44%	18%	28%	1.89	11.29	11.83	22.26	9.16	14.44
SC169	17	72462912	72462993	2%	8%	31%	36%	50%	18%	23%	3.54	12.93	15.09	20.99	7.48	9.78

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC148	6	MAX.chr6.108440646-108440760	-	-	0	-	1	-	-
SC149	3	CHRD	NM_003741	+	0	1364	1	8646	chordin
SC150	14	RIN3	NM_024832	+	0	623	1	79890	Ras and Rab interactor 3
SC151	3	GATA2	NM_001145662;NM_032638;NM_001145661	-	0	4832;10098;5441	0	2624	GATA binding protein 2
SC152	11	MAX.chr11.518925-518978	-	-	0	-	1	-	-
SC153	10	MAX.chr10.102497213-102497267	-	-	0	-	1	-	-
SC154	20	DIDO1	NM_033081;NM_001193369;NM_022105;NM_080797;NM_001193370;NM_080796	-	0	8612;-2789;8612;8612;-2789;-2789	1	11083	death inducer-obliterator 1
SC155	12	CACNA2D4	NM_172364	-	0	121304	1	93589	calcium channel, voltage-dependent, alpha 2/delta subunit 4
SC156	19	MAX.chr19.15695457-15695579	-	-	0	-	1	-	-
SC157	1	MAX.chr1.17215983-17216025	-	-	0	-	1	-	-
SC158	5	ANKRD33B	NM_001164440	+	0	975	1	651746	ankyrin repeat domain 33B
SC159	17	BTBD17	NM_001080466	-	1	4697	1	388419	BTB (POZ) domain containing 17
SC160	5	MAX.chr5.72732853-72732891	-	-	0	-	1	-	-
SC161	17	ERBB2	NM_001005862;NM_004448	+	0	12341;480	1	2064	v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian)
SC162	2	FAM59B	NM_001191033;NM_001168241	+	1	4137;11762	1	150946	family with sequence similarity 59, member B
SC163	19	LOC284454	NR_036515	-	0	-2905	0	284454	hypothetical LOC284454
SC164	1	MAX.chr1.203236695-203236828	-	-	0	-	0	-	-
SC165	16	VAC14	NM_018052	-	0	63380	0	55697	Vac14 homolog (S. cerevisiae)
SC166	12	B4GALNT1	NM_001478	-	1	5362	1	2583	beta-1,4-N-acetyl-galactosaminyl transferase 1
SC167	16	MAX.chr16.54971076-54971118	-	-	0	-	1	-	-
SC168	14	CLEC14A	NM_175060	-	1	888	1	161198	C-type lectin domain family 14, member A
SC169	17	CD300A	NM_007261	+	0	391	0	11314	CD300a molecule

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC170	6	137809402	137809447	3%	6%	15%	26%	52%	17%	30%	2.25	5.60	9.81	20.00	6.36	11.62
SC171	1	2165937	2166058	0%	0%	12%	17%	64%	6%	16%	1.37	45.20	63.35	232.04	20.22	59.05
SC172	5	1295194	1295314	0%	1%	8%	12%	26%	2%	14%	3.69	41.84	62.47	142.53	9.07	77.02
SC173	2	162275439	162275474	1%	3%	10%	14%	48%	9%	36%	5.70	18.84	25.90	90.26	17.46	68.42
SC174	10	101290842	101290919	1%	4%	15%	18%	36%	14%	23%	4.38	16.87	19.48	38.83	15.36	24.73
SC175	1	234812135	234812224	1%	2%	10%	6%	29%	5%	28%	1.88	11.68	7.82	35.70	5.65	34.50
SC176	11	19263923	19264036	3%	5%	7%	20%	60%	25%	17%	1.74	2.61	7.65	22.68	9.43	6.62
SC177	9	140172812	140172891	1%	3%	15%	19%	59%	14%	22%	3.41	18.18	22.23	69.29	17.02	25.45
SC178	5	10565521	10565594	2%	4%	19%	22%	58%	10%	25%	2.38	12.25	14.08	37.71	6.27	15.94
SC179	4	185089599	185089691	1%	1%	1%	3%	21%	2%	13%	0.84	1.68	4.37	28.95	2.54	18.51
SC180	16	54970281	54970313	2%	6%	8%	18%	62%	27%	23%	2.59	3.50	8.08	27.15	11.87	9.87
SC181	17	66596248	66596303	1%	3%	3%	5%	29%	4%	14%	2.13	2.04	4.21	22.21	3.41	11.14
SC182	16	66462102	66462185	1%	5%	18%	10%	43%	10%	9%	4.54	17.45	9.65	41.88	10.12	8.33
SC183	1	32237619	32237654	1%	5%	32%	35%	60%	14%	28%	7.65	53.16	57.57	100.17	23.82	46.87
SC184	8	38614991	38615104	2%	2%	3%	5%	49%	2%	40%	1.28	1.74	3.01	29.81	1.51	24.22
SC185	9	100610931	100611004	2%	4%	17%	22%	47%	23%	37%	2.05	8.53	10.73	22.99	11.47	18.03
SC186	15	63795434	63795521	0%	3%	2%	9%	61%	14%	12%	7.47	4.71	19.91	136.24	30.85	26.64
SC187	9	79627082	79627152	1%	4%	9%	18%	64%	23%	69%	5.03	10.01	21.19	74.36	26.24	80.47
SC188	17	75315486	75315625	1%	1%	3%	4%	61%	4%	12%	0.78	2.49	2.93	50.04	3.41	10.23
SC189	2	43452148	43452243	0%	6%	8%	16%	70%	12%	23%	25.05	34.78	66.17	297.87	50.55	97.10
SC190	12	4382022	4382106	0%	1%	6%	5%	11%	1%	9%	16.03	84.94	83.33	170.21	19.51	137.90
SC191	22	42353835	42353881	0%	4%	2%	12%	70%	13%	30%	9.74	4.94	29.71	168.53	31.52	71.80
SC192	19	39993602	39993646	1%	1%	7%	9%	74%	8%	41%	1.30	6.55	8.24	66.85	6.94	37.03

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC170	6	MAX.chr6.137809402-137809447	-	-	0	-	1	-	-
SC171	1	SKI	NM_003036	+	0	5804	0	6497	v-ski sarcoma viral oncogene homolog (avian)
SC172	5	TERT	NM_001193376;NM_198253	-	0	-32;-32	1	7015	telomerase reverse transcriptase
SC173	2	TBR1	NM_006593	+	1	2820	1	10716	T-box, brain, 1
SC174	10	NKX2-3	NM_145285	+	0	-1847	1	159296	NK2 transcription factor related, locus 3 (Drosophila)
SC175	1	MAX.chr1.234812135-234812224	-	-	0	-	0	-	-
SC176	11	E2F8	NM_024680	-	0	-1416	1	79733	E2F transcription factor 8
SC177	9	C9orf167	NM_017723	+	0	533	1	54863	chromosome 9 open reading frame 167
SC178	5	ANKRD33B	NM_001164440	+	0	1087	1	651746	ankyrin repeat domain 33B
SC179	4	ENPP6	NM_153343	-	0	49515	0	133121	ectonucleotide pyrophosphatase/phosphodiesterase 6
SC180	16	MAX.chr16.54970281-54970313	-	-	0	-	0	-	-
SC181	17	FAM20A	NM_017565	-	0	847	1	54757	family with sequence similarity 20, member A
SC182	16	BEAN1	NM_001178020;NM_001197225;NM_001136106;NM_001197224	+	0	1287;1287;1287;1287	1	146227	brain expressed, associated with NEDD4, 1
SC183	1	MAX.chr1.32237619-32237654	-	-	0	-	0	-	-
SC184	8	TACC1	NM_001146216	+	0	29288	1	6867	transforming, acidic coiled-coil containing protein 1
SC185	9	FOXE1	NM_004473	+	0	-4605	1	2304	forkhead box E1 (thyroid transcription factor 2)
SC186	15	USP3	NM_006537	+	0	-1375	0	9960	ubiquitin specific peptidase 3
SC187	9	MAX.chr9.79627082-79627152	-	-	0	-	0	-	-
SC188	17	9-Sep	NM_006640;NM_001113492;NM_001113491	+	0	-110;31514;37995	0	10801	septin 9
SC189	2	LOC100129726	NR_027251	+	1	-2201	1	100129726	hypothetical LOC100129726
SC190	12	CCND2	NM_001759	+	0	-879	1	894	cyclin D2
SC191	22	LOC339674	NR_024355	+	1	5645	1	339674	hypothetical LOC339674
SC192	19	DLL3	NM_016941;NM_203486	+	1	4046;4046	1	10683	delta-like 3 (Drosophila)

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC193	5	10333588	10333742	1%	1%	4%	6%	38%	5%	25%	1.25	5.09	8.20	51.82	6.55	34.37
SC194	14	24808727	24808770	1%	4%	3%	11%	32%	7%	8%	5.37	4.89	16.21	46.37	10.19	11.60
SC195	1	32237695	32237880	1%	3%	22%	25%	44%	10%	14%	2.78	22.43	25.97	45.37	10.13	14.59
SC196	14	95234712	95234849	2%	4%	8%	18%	43%	8%	27%	2.56	5.27	11.66	27.79	5.40	17.45
SC197	19	14667596	14667671	0%	1%	1%	1%	24%	5%	5%	1.51	2.28	3.41	60.37	13.55	13.44
SC198	15	28352738	28352817	1%	5%	12%	21%	58%	11%	21%	3.25	8.34	14.55	40.37	7.29	14.34
SC199	16	10480178	10480238	0%	1%	2%	4%	19%	3%	28%	6.60	11.96	26.48	116.02	15.18	166.59
SC200	22	19742789	19742857	2%	5%	10%	14%	47%	17%	13%	2.51	5.00	7.01	24.29	8.86	6.63
SC201	5	42995328	42995393	0%	4%	12%	28%	50%	30%	55%	24.55	77.50	185.42	325.38	198.95	362.13
SC202	5	42993267	42993312	0%	6%	15%	22%	45%	18%	55%	20.06	48.10	71.60	147.07	59.74	178.70
SC203	12	58021483	58021536	1%	8%	12%	26%	69%	17%	29%	11.91	18.78	39.87	106.60	27.11	44.27
SC204	12	25055873	25055997	0%	1%	10%	22%	29%	12%	40%	4.32	36.00	77.51	103.02	44.17	140.87
SC205	2	43451937	43452012	1%	4%	5%	8%	57%	9%	13%	5.93	7.65	11.58	84.17	13.31	19.60
SC206	6	1620122	1620172	1%	7%	6%	15%	72%	41%	31%	5.87	5.36	12.51	60.18	34.60	25.88
SC207	9	37037883	37037949	1%	6%	17%	14%	66%	5%	56%	3.77	11.52	9.77	44.81	3.27	38.35
SC208	5	10333749	10333885	1%	1%	2%	6%	43%	6%	23%	1.04	1.70	5.02	36.10	5.17	18.80
SC209	8	55367295	55367420	1%	3%	10%	21%	39%	10%	42%	3.21	9.56	19.77	35.89	9.58	38.62
SC210	2	100721771	100721847	1%	1%	4%	6%	26%	1%	5%	1.18	4.71	7.92	33.23	1.92	6.91
SC211	13	53313456	53313530	1%	5%	16%	24%	46%	9%	47%	3.27	10.97	16.69	31.99	6.61	32.97
SC212	2	95401460	95401490	2%	5%	25%	30%	53%	30%	59%	3.06	14.07	17.04	30.20	16.81	33.42
SC213	2	177017228	177017277	2%	5%	21%	16%	46%	26%	16%	3.06	12.85	10.12	28.49	16.05	9.69
SC214	5	10333423	10333478	2%	6%	11%	25%	48%	15%	30%	2.97	5.20	11.92	22.61	7.11	14.28
SC215	9	139024776	139024933	2%	4%	12%	15%	48%	5%	32%	1.71	5.77	6.96	22.15	2.28	14.73
SC216	15	68125482	68125550	1%	4%	15%	20%	52%	17%	26%	3.44	13.77	18.50	47.09	15.83	23.80
SC217	17	72352858	72352916	2%	4%	9%	14%	52%	7%	42%	1.79	4.18	6.40	23.41	3.14	18.93

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC193	5	MAX.chr5.10333588-10333742	-	-	0	-	1	-	-
SC194	14	ADCY4	NM_139247;NM_001198592;NM_001198568	-	1	-4450;-4450;-4450	1	196883	adenylate cyclase 4
SC195	1	MAX.chr1.32237695-32237880	-	-	0	-	0	-	-
SC196	14	GSC	NM_173849	-	1	1787	1	145258	goosecoid homeobox
SC197	19	TECR	NM_138501	+	0	27215	0	9524	trans-2,3-enoyl-CoA reductase
SC198	15	MAX.chr15.28352738-28352817	-	-	0	-	1	-	-
SC199	16	MAX.chr16.10480178-10480238	-	-	0	-	1	-	-
SC200	22	TBX1	NM_005992;NM_080647;NM_080646	+	0	-1436;-1436;-1436	0	6899	T-box 1
SC201	5	MAX.chr5.42995328-42995393	-	-	0	-	1	-	-
SC202	5	MAX.chr5.42993267-42993312	-	-	0	-	0	-	-
SC203	12	B4GALNT1	NM_001478	-	1	5502	1	2583	beta-1,4-N-acetyl-galactosaminyl transferase 1
SC204	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46520;46520;-551;46520;136	1	586	branched chain amino-acid transaminase 1, cytosolic
SC205	2	LOC100129726	NR_027251	+	1	-2412	1	100129726	hypothetical LOC100129726
SC206	6	MAX.chr6.1620122-1620172	-	-	0	-	1	-	-
SC207	9	PAX5	NM_016734	-	0	-3407	1	5079	paired box 5
SC208	5	MAX.chr5.10333749-10333885	-	-	0	-	1	-	-
SC209	8	SOX17	NM_022454	+	0	-3199	1	64321	SRY (sex determining region Y)-box 17
SC210	2	AFF3	NM_002285;NM_001025108	-	0	37266;274	0	3899	AF4/FMR2 family, member 3
SC211	13	LECT1	NM_007015;NM_001011705	-	0	491;491	1	11061	leukocyte cell derived chemotaxin 1
SC212	2	MAX.chr2.95401460-95401490	-	-	0	-	1	-	-
SC213	2	HOXD4	NM_014621	+	0	1116	0	3233	homeobox D4
SC214	5	MAX.chr5.10333423-10333478	-	-	0	-	1	-	-
SC215	9	MAX.chr9.139024776-139024933	-	-	0	-	1	-	-
SC216	15	SKOR1	NM_001031807	+	0	7542	0	390598	SKI family transcriptional corepressor 1
SC217	17	BTBD17	NM_001080466	-	1	5100	1	388419	BTB (POZ) domain containing 17

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC218	6	2903614	2903705	0%	1%	2%	13%	21%	2%	1%	1.58	3.90	28.42	45.90	4.89	1.60
SC219	1	108506611	108506701	0%	1%	8%	10%	21%	4%	19%	6.54	35.25	44.05	91.51	18.41	82.35
SC220	10	124910491	124910540	2%	4%	9%	32%	57%	15%	24%	2.62	5.68	19.94	35.80	9.12	15.29
SC221	5	100240192	100240273	0%	5%	8%	11%	63%	13%	10%	41.36	62.03	86.85	479.51	95.95	77.29
SC222	14	60976754	60976830	3%	6%	31%	26%	64%	28%	64%	2.07	10.76	9.12	22.28	9.82	22.57
SC223	1	221052041	221052157	1%	5%	11%	27%	39%	5%	29%	7.04	15.55	37.83	54.48	7.03	40.64
SC224	12	25055634	25055804	1%	3%	14%	27%	30%	17%	50%	5.65	23.18	44.87	51.11	28.60	83.14
SC225	12	4140345	4140422	0%	1%	2%	3%	11%	1%	4%	2.43	6.24	9.50	39.21	4.13	15.23
SC226	1	47698051	47698082	1%	4%	24%	18%	51%	16%	48%	4.25	22.37	17.39	47.95	15.43	45.65
SC227	2	30453799	30453967	0%	2%	15%	17%	21%	7%	22%	7.67	47.98	55.02	69.82	24.31	72.43
SC228	2	26408004	26408041	1%	2%	3%	9%	44%	15%	17%	3.91	5.50	18.17	87.75	29.51	34.54
SC229	4	8859253	8859363	0%	1%	4%	14%	14%	7%	18%	3.88	9.54	36.29	35.88	18.16	46.41
SC230	10	52177880	52177955	0%	0%	1%	1%	35%	7%	7%	0.41	1.18	2.25	71.20	13.46	14.52
SC231	8	99960542	99960654	1%	3%	10%	20%	44%	29%	24%	2.42	9.58	18.79	40.90	26.85	22.50
SC232	12	25056183	25056246	1%	5%	19%	37%	54%	29%	64%	4.03	13.60	27.03	39.72	21.36	47.09
SC233	14	85996211	85996331	1%	2%	6%	5%	46%	6%	37%	1.50	4.65	3.78	34.78	4.76	28.13
SC234	14	78108294	78108420	1%	3%	2%	13%	42%	6%	25%	1.94	1.38	8.93	29.33	4.53	17.37
SC235	6	26273748	26273836	2%	3%	4%	28%	39%	9%	34%	1.84	2.40	17.99	25.11	5.70	22.17
SC236	17	72209156	72209196	0%	1%	1%	3%	27%	1%	9%	2.15	1.82	7.56	73.90	3.66	25.72
SC237	6	137809670	137809791	2%	4%	11%	18%	44%	10%	18%	1.83	5.96	9.48	22.78	5.22	9.41
SC238	6	28175437	28175586	1%	2%	7%	16%	23%	6%	14%	3.23	9.57	21.74	31.93	8.81	19.25

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC218	6	SERPINB9	NM_004155	-	0	-69	1	5272	serpin peptidase inhibitor, clade B (ovalbumin), member 9
SC219	1	VAV3	NM_006113	-	0	934	0	10451	vav 3 guanine nucleotide exchange factor
SC220	10	BUB3	NM_001007793;NM_004725	+	0	-3268;-3268	1	9184	budding uninhibited by benzimidazoles 3 homolog (yeast)
SC221	5	ST8SIA4	NM_005668;NM_175052	-	0	-1205;-1222	0	7903	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 4
SC222	14	SIX6	NM_007374	+	0	817	1	4990	SIX homeobox 6
SC223	1	HLX	NM_021958	+	0	-701	1	3142	H2.0-like homeobox
SC224	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46759;46759;-312;46759;375	1	586	branched chain amino-acid transaminase 1, cytosolic
SC225	12	MAX.chr12.4140345-4140422	-	-	0	-	0	-	-
SC226	1	TAL1	NM_003189	-	0	-2608	1	6886	T-cell acute lymphocytic leukemia 1
SC227	2	LBH	NM_030915	+	0	-597	1	81606	limb bud and heart development homolog (mouse)
SC228	2	FAM59B	NM_001191033;NM_001168241	+	1	4420;12045	1	150946	family with sequence similarity 59, member B
SC229	4	MAX.chr4.8859253-8859363	-	-	0	-	1	-	-
SC230	10	SGMS1	NM_147156	-	0	205857	1	259230	sphingomyelin synthase 1
SC231	8	OSR2	NM_001142462;NM_053001	+	0	3912;3912	1	116039	odd-skipped related 2 (Drosophila)
SC232	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46210;46210;-861;46210;-174	1	586	branched chain amino-acid transaminase 1, cytosolic
SC233	14	FLRT2	NM_013231	+	0	-276	0	23768	fibronectin leucine rich transmembrane protein 2
SC234	14	MAX.chr14.78108294-78108420	-	-	0	-	1	-	-
SC235	6	HIST1H3G	NM_003534	-	0	-2136	0	8355	histone cluster 1, H3g
SC236	17	MGC16275	NR_026914	-	1	304	0	85001	hypothetical protein MGC16275
SC237	6	MAX.chr6.137809670-137809791	-	-	0	-	1	-	-
SC238	6	MAX.chr6.28175437-28175586	-	-	0	-	1	-	-

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC239	4	175135937	175135997	2%	4%	5%	22%	35%	7%	37%	2.24	3.27	13.71	22.01	4.52	23.04
SC240	9	94444034	94444124	2%	7%	6%	17%	44%	14%	48%	3.20	2.74	8.49	21.68	6.85	23.33
SC241	11	19263815	19263912	2%	4%	5%	16%	54%	17%	13%	2.43	2.71	9.32	31.54	10.14	7.44
SC242	22	31481121	31481159	0%	1%	5%	9%	59%	6%	28%	2.63	15.70	26.55	170.11	15.93	81.64
SC243	6	27059752	27059869	0%	2%	4%	4%	38%	3%	40%	4.64	10.35	10.26	104.70	8.90	110.25
SC244	7	22539866	22539943	1%	1%	3%	3%	13%	5%	10%	1.05	4.46	5.80	20.78	8.83	16.46
SC245	11	518684	518729	0%	0%	0%	0%	24%	6%	3%	1.94	2.51	2.30	138.27	36.98	15.71
SC246	22	31481428	31481510	1%	2%	12%	10%	50%	6%	18%	1.46	8.29	6.87	34.17	4.41	12.41
SC247	17	48636503	48636608	1%	2%	3%	5%	21%	5%	6%	2.71	3.51	6.21	24.43	5.92	7.43
SC248	10	26727989	26728120	0%	2%	4%	6%	19%	2%	24%	11.37	28.27	43.79	139.11	15.00	180.22
SC249	7	2558594	2558664	1%	1%	6%	5%	47%	3%	16%	1.38	5.78	5.37	45.60	2.76	15.89
SC250	9	94444127	94444240	1%	3%	3%	14%	48%	12%	44%	2.41	2.74	12.60	43.62	10.94	39.33
SC251	17	37321144	37321212	0%	1%	2%	3%	16%	6%	4%	1.92	6.27	9.79	58.67	21.36	16.09
SC252	7	155302555	155302648	1%	2%	13%	16%	29%	11%	40%	2.09	12.76	16.09	29.56	10.56	39.78
SC253	1	156863477	156863554	1%	3%	24%	19%	32%	14%	13%	2.42	20.38	15.89	26.82	11.70	10.87
SC254	1	40236941	40237022	3%	5%	30%	35%	74%	22%	77%	1.58	9.71	11.35	24.36	7.07	25.14
SC255	2	200328930	200328964	2%	6%	13%	20%	42%	14%	45%	2.95	6.68	10.18	21.87	7.45	23.16
SC256	16	4422078	4422139	0%	0%	0%	0%	49%	1%	17%	2.62	1.65	2.25	369.94	8.01	131.13
SC257	19	13950379	13950468	1%	3%	9%	4%	37%	9%	16%	2.62	9.53	4.40	37.32	8.87	16.23
SC258	1	32410360	32410416	1%	0%	1%	2%	26%	8%	25%	0.49	2.33	3.19	48.05	14.55	45.91
SC259	17	58499109	58499183	0%	1%	1%	5%	35%	18%	8%	4.22	4.26	17.93	124.48	63.79	28.58

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC239	4	MAX.chr4.175135937-175135997	-	-	0	-	0	-	-
SC240	9	MAX.chr9.94444034-94444124	-	-	0	-	0	-	-
SC241	11	E2F8	NM_024680	-	0	-1308	1	79733	E2F transcription factor 8
SC242	22	SMTN	NM_134269;NM_006932;NM_134270	+	0	3817;3817;3817	1	6525	smoothelin
SC243	6	MAX.chr6.27059752-27059869	-	-	0	-	0	-	-
SC244	7	MGC87042	NM_207342;NM_001164460	-	1	35;35	1	256227	STEAP family protein MGC87042
SC245	11	MAX.chr11.518684-518729	-	-	0	-	1	-	-
SC246	22	SMTN	NM_134269;NM_006932;NM_134270	+	0	4124;4124;4124	0	6525	smoothelin
SC247	17	CACNA1G	NM_018896;NM_198376;NM_198387;NM_198388;NM_198378;NM_198380;NM_198385;NM_198397;NM_198386;NM_198383;NM_198377;NM_198379;NM_198396;NM_198382;NM_198384	+	0	-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945;-1945	1	8913	calcium channel, voltage-dependent, T type, alpha 1G subunit
SC248	10	APBB1IP	NM_019043	+	0	724	1	54518	amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
SC249	7	LFNG	NM_001040167;NM_001166355;NM_001040168;NM_002304	+	0	-884;6432;-884;1098	1	3955	LFNG O-fucosylpeptide 3-beta-N-acetylglucosaminyltransferase
SC250	9	MAX.chr9.94444127-94444240	-	-	0	-	0	-	-
SC251	17	ARL5C	NM_001143968	-	0	1270	0	390790	ADP-ribosylation factor-like 5C
SC252	7	CNPY1	NM_001103176	-	0	23984	1	285888	canopy 1 homolog (zebrafish)
SC253	1	PEAR1	NM_001080471	+	0	-45	1	375033	platelet endothelial aggregation receptor 1
SC254	1	BMP8B	NM_001720	-	1	17592	1	656	bone morphogenetic protein 8b
SC255	2	FLJ32063	NR_026830	+	0	-3890	1	150538	hypothetical LOC150538
SC256	16	CORO7	NM_024535	-	0	44561	1	79585	coronin 7
SC257	19	LOC284454	NR_036515	-	0	-3276	0	284454	hypothetical LOC284454
SC258	1	MAX.chr1.32410360-32410416	-	-	0	-	1	-	-
SC259	17	C17orf64	NM_181707	+	0	-755	1	124773	chromosome 17 open reading frame 64

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC260	12	25102040	25102115	1%	1%	2%	7%	30%	6%	46%	0.98	1.54	6.05	24.87	5.12	37.73
SC261	12	25101810	25101893	0%	0%	0%	6%	24%	4%	40%	1.38	1.62	25.21	97.73	16.47	163.70
SC262	5	169064211	169064314	0%	1%	9%	15%	30%	2%	22%	3.71	29.12	46.14	93.41	5.35	71.09
SC263	7	5635750	5635844	1%	4%	23%	13%	35%	5%	12%	3.30	18.65	10.10	28.57	3.82	9.54
SC264	20	44746681	44746768	1%	2%	5%	6%	25%	3%	7%	1.71	5.43	6.36	26.47	3.23	6.91
SC265	17	70216308	70216394	2%	4%	17%	25%	48%	16%	70%	1.74	8.10	12.23	23.33	7.58	34.05
SC266	2	25439185	25439264	0%	1%	10%	11%	31%	3%	7%	2.31	25.89	26.41	77.49	6.89	18.37
SC267	19	16022754	16022843	1%	3%	26%	23%	40%	19%	40%	2.77	20.59	18.27	32.36	15.43	32.48
SC268	6	157557573	157557649	2%	3%	5%	25%	66%	16%	33%	1.63	2.76	12.83	34.25	8.20	17.06
SC269	12	54812386	54812467	1%	2%	12%	16%	28%	9%	9%	1.67	11.36	14.63	26.03	8.59	8.08
SC270	17	36204463	36204540	2%	4%	6%	12%	44%	9%	44%	2.57	3.62	7.26	25.84	5.35	26.21
SC271	11	64108279	64108358	0%	4%	16%	16%	40%	9%	21%	13.17	51.71	50.68	128.96	27.78	69.11
SC272	5	10563501	10563540	1%	4%	11%	20%	30%	3%	29%	4.01	11.13	19.49	30.32	2.98	28.82
SC273	12	22486883	22487008	0%	1%	5%	11%	28%	12%	43%	5.71	19.90	40.52	108.42	47.79	164.13
SC274	19	58238816	58238942	0%	1%	8%	15%	24%	17%	33%	8.09	94.63	175.66	277.57	203.93	390.90
SC275	9	124132453	124132500	0%	0%	0%	0%	11%	0%	19%	0.40	1.95	2.34	49.53	1.58	87.46
SC276	5	37834916	37835022	1%	5%	9%	28%	55%	37%	42%	4.35	7.78	25.11	49.26	33.19	37.95
SC277	1	61519406	61519521	1%	3%	3%	9%	32%	10%	19%	4.49	4.18	12.66	46.99	13.78	28.13
SC278	19	1467153	1467216	2%	4%	11%	25%	53%	15%	42%	2.08	5.76	12.88	26.95	7.57	21.46
SC279	3	186648031	186648076	1%	1%	2%	4%	23%	6%	2%	1.18	2.18	3.54	22.27	5.47	1.72

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC260	12	BCAT1	NM_001178092;NM_005504;NM_001178091	-	1	353;353;353	1	586	branched chain amino-acid transaminase 1, cytosolic
SC261	12	BCAT1	NM_001178092;NM_005504;NM_001178091	-	0	583;583;583	1	586	branched chain amino-acid transaminase 1, cytosolic
SC262	5	DOCK2	NM_004946	+	0	-39	0	1794	dedicator of cytokinesis 2
SC263	7	FSCN1	NM_003088	+	0	3297	0	6624	fascin homolog 1, actin-bundling protein (Strongylocentrotus purpuratus)
SC264	20	CD40	NM_001250;NM_152854	+	0	-224;-224	0	958	CD40 molecule, TNF receptor superfamily member 5
SC265	17	MAX.chr17.70216308-70216394	-	-	0	-	0	-	-
SC266	2	MAX.chr2.25439185-25439264	-	-	0	-	1	-	-
SC267	19	MAX.chr19.16022754-16022843	-	-	0	-	1	-	-
SC268	6	MAX.chr6.157557573-157557649	-	-	0	-	1	-	-
SC269	12	ITGA5	NM_002205	-	0	664	0	3678	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
SC270	17	LOC284100	NR_024178	-	0	39900	0	284100	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, epsilon polypeptide pseudogene
SC271	11	CCDC88B	NM_032251	+	0	590	0	283234	coiled-coil domain containing 88B
SC272	5	ANKRD33B	NM_001164440	+	0	-933	1	651746	ankyrin repeat domain 33B
SC273	12	ST8SIA1	NM_003034	-	0	765	1	6489	ST8 alpha-N-acetyl-neuraminide alpha-2,8-sialyltransferase 1
SC274	19	ZNF671	NM_024833	-	1	179	1	79891	zinc finger protein 671
SC275	9	STOM	NM_198194;NM_004099	-	1	92;92	1	2040	stomatin
SC276	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	1	1013;1013;4866;677	1	2668	glial cell derived neurotrophic factor
SC277	1	MAX.chr1.61519406-61519521	-	-	0	-	1	-	-
SC278	19	APC2	NM_005883	+	1	17006	1	10297	adenomatosis polyposis coli 2
SC279	3	ST6GAL1	NM_173216;NM_173217	+	0	-283;-283	0	6480	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC280	8	65490049	65490112	2%	5%	6%	11%	49%	12%	38%	2.13	2.63	4.76	21.73	5.34	17.03
SC281	9	96713572	96713746	1%	2%	1%	6%	49%	9%	13%	1.43	1.30	5.34	45.71	8.18	12.40
SC282	1	110627121	110627221	1%	3%	7%	25%	39%	7%	33%	2.37	6.55	22.73	36.30	6.54	30.81
SC283	21	44494923	44494962	1%	4%	13%	10%	41%	12%	16%	3.28	10.92	8.29	34.30	9.61	13.41
SC284	17	47073394	47073480	1%	3%	12%	25%	31%	5%	30%	2.03	8.66	18.20	22.07	3.33	21.89
SC285	9	96715505	96715595	2%	5%	27%	26%	56%	22%	13%	2.12	12.92	12.09	26.40	10.29	6.21
SC286	1	61519679	61519759	0%	1%	2%	8%	31%	8%	13%	5.20	10.06	37.79	143.41	37.27	60.12
SC287	6	157557374	157557528	1%	1%	1%	11%	36%	9%	15%	2.32	2.11	18.24	61.73	15.37	26.63
SC288	1	214158912	214158969	1%	4%	20%	33%	50%	18%	46%	3.39	17.95	29.64	45.85	16.83	41.54
SC289	6	1624797	1624838	1%	5%	13%	9%	39%	13%	24%	4.95	13.39	9.22	38.66	12.89	24.01
SC290	6	99295996	99296069	2%	5%	12%	20%	54%	22%	18%	2.17	4.91	8.25	22.18	8.83	7.46
SC291	6	6004298	6004338	1%	5%	21%	40%	58%	34%	61%	7.31	33.74	64.23	93.83	54.50	99.42
SC292	2	73147720	73147790	1%	3%	24%	36%	50%	28%	59%	3.25	26.10	38.89	54.76	30.96	64.51
SC293	1	248020671	248020722	1%	4%	31%	40%	63%	33%	63%	3.31	23.46	30.04	47.26	24.66	47.74
SC294	17	43339354	43339495	1%	1%	5%	15%	39%	17%	18%	0.97	6.27	17.23	44.90	19.79	20.18
SC295	13	53421299	53421350	2%	6%	30%	32%	64%	24%	65%	3.20	16.30	17.38	34.40	12.80	34.96
SC296	2	73147853	73147982	1%	5%	23%	30%	41%	25%	57%	3.39	16.03	20.50	28.49	17.46	39.34
SC297	6	137244467	137244587	3%	6%	16%	23%	55%	22%	55%	2.34	6.29	9.20	21.68	8.65	21.76
SC298	1	208132590	208132681	1%	7%	11%	33%	58%	30%	46%	6.30	9.55	28.98	51.43	26.34	41.25
SC299	5	37834716	37834762	2%	4%	16%	25%	51%	36%	36%	2.51	9.42	14.77	30.36	21.39	21.50
SC300	19	10406234	10406282	1%	4%	6%	17%	42%	16%	44%	6.91	11.83	31.08	76.40	29.79	80.36
SC301	9	96715384	96715473	1%	3%	19%	19%	53%	19%	8%	2.78	16.47	17.08	47.26	17.24	6.84
SC302	20	21493456	21493605	1%	3%	13%	19%	48%	6%	16%	2.82	10.65	15.29	39.13	4.59	13.02
SC303	1	61519535	61519667	0%	1%	2%	9%	35%	8%	17%	2.03	3.47	17.58	71.29	15.47	34.29
SC304	19	48983840	48983937	1%	4%	16%	18%	58%	15%	31%	2.99	12.22	13.83	44.21	11.76	23.49

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC280	8	BHLHE22	NM_152414	+	0	-2764	0	27319	basic helix-loop-helix family, member e22
SC281	9	MAX.chr9.96713572-96713746	-	-	0	-	1	-	-
SC282	1	MAX.chr1.110627121-110627221	-	-	0	-	1	-	-
SC283	21	CBS	NM_001178008;NM_001178009;NM_000071	-	0	1549;1549;1117	1	875	cystathionine-beta-synthase
SC284	17	IGF2BP1	NM_006546;NM_001160423	+	0	-1379;-1379	1	10642	insulin-like growth factor 2 mRNA binding protein 1
SC285	9	BARX1	NM_021570	-	0	2103	1	56033	BARX homeobox 1
SC286	1	MAX.chr1.61519679-61519759	-	-	0	-	1	-	-
SC287	6	MAX.chr6.157557374-157557528	-	-	0	-	1	-	-
SC288	1	PROX1	NM_002763	+	0	-2947	1	5629	prospero homeobox 1
SC289	6	GMDS	NM_001500	-	0	621049	1	2762	GDP-mannose 4,6-dehydratase
SC290	6	MAX.chr6.99295996-99296069	-	-	0	-	1	-	-
SC291	6	NRN1	NM_016588	-	0	3335	1	51299	neuritin 1
SC292	2	EMX1	NM_004097	+	0	3117	1	2016	empty spiracles homeobox 1
SC293	1	TRIM58	NM_015431	+	1	171	1	25893	tripartite motif-containing 58
SC294	17	C17orf46	NM_152343	-	0	125	1	124783	chromosome 17 open reading frame 46
SC295	13	PCDH8	NM_002590;NM_032949	-	1	1475;1475	1	5100	protocadherin 8
SC296	2	EMX1	NM_004097	+	0	3250	1	2016	empty spiracles homeobox 1
SC297	6	SLC35D3	NM_001008783	+	0	1066	1	340146	solute carrier family 35, member D3
SC298	1	MAX.chr1.208132590-208132681	-	-	0	-	1	-	-
SC299	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	0	1213;1213;5066;877	1	2668	glial cell derived neurotrophic factor
SC300	19	ICAM5	NM_003259	+	1	5580	1	7087	intercellular adhesion molecule 5, telencephalin
SC301	9	BARX1	NM_021570	-	1	2224	1	56033	BARX homeobox 1
SC302	20	NKX2-2	NM_002509	-	0	1208	1	4821	NK2 homeobox 2
SC303	1	MAX.chr1.61519535-61519667	-	-	0	-	1	-	-
SC304	19	CYTH2	NM_004228;NM_017457	+	1	11376;11376	1	9266	cytohesin 2

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC305	6	108490524	108490584	1%	2%	11%	18%	30%	18%	3%	1.88	9.31	15.77	25.30	15.66	2.40
SC306	5	149792249	149792285	0%	3%	1%	5%	29%	13%	17%	17.71	6.75	24.08	147.62	64.65	85.42
SC307	6	44119711	44119872	0%	1%	1%	7%	14%	5%	10%	2.29	1.94	17.47	34.31	13.65	24.44
SC308	19	38182957	38183121	1%	2%	18%	13%	26%	17%	28%	1.78	16.97	11.59	24.34	15.34	25.69
SC309	22	46263416	46263515	2%	4%	4%	9%	47%	24%	19%	1.60	1.87	4.14	21.04	10.94	8.60
SC310	1	248020410	248020517	2%	4%	20%	28%	42%	15%	46%	2.26	11.28	15.61	22.96	8.22	25.33
SC311	17	43339264	43339345	1%	1%	4%	11%	41%	12%	11%	1.45	8.50	22.19	82.48	23.48	21.17
SC312	5	134879362	134879483	0%	1%	5%	13%	35%	5%	17%	3.15	10.56	29.38	80.54	10.94	39.68
SC313	10	101300100	101300155	1%	3%	10%	16%	24%	5%	7%	3.69	13.71	22.13	33.20	6.17	9.10
SC314	5	37834845	37834910	2%	5%	13%	29%	50%	39%	33%	3.22	8.38	18.64	32.37	25.14	21.61
SC315	20	36013131	36013210	0%	2%	4%	4%	20%	7%	11%	9.17	23.33	24.65	124.06	40.85	68.15
SC316	3	16554363	16554496	0%	0%	0%	3%	11%	4%	2%	3.51	1.43	26.47	107.49	36.66	15.44
SC317	12	25056015	25056162	0%	1%	10%	18%	25%	10%	35%	1.51	27.62	50.51	68.55	27.10	95.52
SC318	6	157556780	157556850	1%	3%	3%	17%	49%	11%	11%	2.07	1.73	11.91	33.83	7.40	7.57
SC319	12	122231765	122231829	1%	1%	1%	7%	18%	2%	1%	1.59	2.48	12.95	35.04	3.13	2.54
SC320	3	182897149	182897232	1%	1%	3%	4%	24%	1%	10%	0.83	4.11	5.33	32.87	1.36	13.04
SC321	18	5891056	5891125	2%	4%	12%	29%	49%	9%	36%	2.12	6.28	14.73	24.80	4.33	18.25
SC322	11	76750814	76750881	1%	8%	30%	27%	52%	16%	64%	10.48	41.11	36.46	70.66	22.09	86.31
SC323	19	2251365	2251400	2%	4%	15%	18%	65%	10%	22%	2.23	8.49	10.23	37.40	5.66	12.69

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC305	6	NR2E1	NM_003269	+	0	3310	1	7101	nuclear receptor subfamily 2, group E, member 1
SC306	5	CD74	NM_001025158;NM_004355;NM_001025159	-	1	83;74;74	0	972	CD74 molecule, major histocompatibility complex, class II invariant chain
SC307	6	TMEM63B	NM_018426	+	1	24336	1	55362	transmembrane protein 63B
SC308	19	ZNF781	NM_152605	-	0	259	1	163115	zinc finger protein 781
SC309	22	MAX.chr22.46263416-46263515	-	-	0	-	1	-	-
SC310	1	TRIM58	NM_015431	+	0	-90	1	25893	tripartite motif-containing 58
SC311	17	C17orf46	NM_152343	-	0	215	1	124783	chromosome 17 open reading frame 46
SC312	5	MAX.chr5.134879362-134879483	-	-	0	-	0	-	-
SC313	10	MAX.chr10.101300100-101300155	-	-	0	-	1	-	-
SC314	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	1	1084;1084;4937;748	1	2668	glial cell derived neurotrophic factor
SC315	20	SRC	NM_005417;NM_198291	+	0	40044;38575	1	6714	v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian)
SC316	3	RFTN1	NM_015150	-	0	859	1	23180	raftlin, lipid raft linker 1
SC317	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46378;46378;-693;46378;-6	1	586	branched chain amino-acid transaminase 1, cytosolic
SC318	6	MAX.chr6.157556780-157556850	-	-	0	-	0	-	-
SC319	12	RHOF	NM_019034	-	0	-171	1	54509	ras homolog gene family, member F (in filopodia)
SC320	3	MCF2L2	NM_015078	-	1	248706	1	23101	MCF.2 cell line derived transforming sequence-like 2
SC321	18	TMEM200C	NM_001080209	-	1	1047	1	645369	transmembrane protein 200C
SC322	11	B3GNT6	NM_138706	+	1	5380	1	192134	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 6 (core 3 synthase)
SC323	19	AMH	NM_000479	+	1	2253	1	268	anti-Mullerian hormone

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC324	5	154026853	154026896	1%	5%	5%	12%	38%	14%	7%	7.87	7.72	17.90	58.03	21.03	10.87
SC325	1	167599734	167599813	0%	1%	5%	11%	13%	3%	16%	7.00	23.21	52.79	62.19	15.82	79.50
SC326	18	44526868	44526957	1%	1%	6%	6%	22%	18%	7%	1.31	10.36	9.07	36.70	29.96	12.10
SC327	19	48983735	48983826	2%	5%	20%	17%	47%	10%	25%	1.94	8.78	7.43	20.22	4.38	10.74
SC328	9	139159296	139159348	0%	1%	1%	1%	19%	2%	26%	5.60	6.92	3.81	135.14	13.04	185.99
SC329	5	132083179	132083223	1%	1%	2%	6%	30%	2%	5%	1.53	1.67	6.35	32.19	2.49	5.46
SC330	21	26934474	26934630	0%	1%	9%	10%	22%	4%	20%	5.32	55.57	61.25	138.59	27.77	121.89
SC331	6	106960830	106960925	0%	2%	2%	7%	50%	4%	23%	3.36	4.28	15.50	108.22	9.22	49.69
SC332	7	156701663	156701795	1%	1%	1%	6%	17%	5%	6%	1.70	1.22	7.49	21.77	6.28	8.20
SC333	1	47697788	47697839	1%	4%	15%	16%	35%	12%	52%	4.93	18.42	19.77	42.61	14.00	63.25
SC334	5	77140806	77140873	2%	5%	19%	19%	55%	15%	49%	2.10	8.33	8.10	23.90	6.32	21.13
SC335	15	28352323	28352494	1%	1%	4%	8%	15%	2%	20%	1.47	6.11	12.78	24.57	2.78	31.63
SC336	6	29521540	29521681	1%	5%	5%	22%	41%	17%	23%	3.41	3.62	14.78	27.38	11.77	15.51
SC337	14	92980637	92980695	0%	0%	1%	0%	22%	1%	9%	0.63	3.61	1.60	91.32	3.10	36.73
SC338	9	19127781	19127835	0%	0%	1%	1%	20%	1%	3%	1.93	4.37	3.15	118.01	4.59	17.38
SC339	1	6663497	6663545	0%	1%	1%	1%	15%	4%	8%	2.59	3.84	3.13	64.79	19.15	35.96
SC340	1	2136959	2137098	0%	0%	1%	1%	12%	2%	2%	1.41	2.04	4.52	37.03	6.29	7.19
SC341	21	36042030	36042110	1%	2%	4%	22%	41%	13%	55%	1.88	3.80	23.37	43.13	13.73	57.60
SC342	3	170137371	170137439	2%	5%	15%	34%	49%	33%	62%	2.78	9.08	20.26	29.02	19.46	36.27
SC343	6	158243942	158243981	1%	1%	1%	2%	33%	2%	13%	1.38	1.60	2.72	36.48	1.68	14.51
SC344	12	125534296	125534343	0%	1%	1%	10%	29%	5%	0%	3.25	1.46	24.52	68.16	12.02	0.91
SC345	12	1906061	1906126	0%	0%	0%	8%	23%	0%	4%	0.76	0.41	20.08	57.07	0.76	9.54
SC346	10	119312919	119312997	1%	3%	15%	26%	28%	5%	11%	2.57	12.66	22.08	23.61	4.33	9.25
SC347	13	25321012	25321114	1%	2%	2%	6%	26%	16%	26%	2.12	2.30	7.65	31.63	19.72	31.63
SC348	9	94183673	94183710	1%	1%	9%	10%	24%	6%	20%	1.61	10.63	12.61	29.26	7.25	24.73

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC324	5	MAX.chr5.154026853-154026896	-	-	0	-	1	-	-
SC325	1	RCSD1	NM_052862	+	0	261	1	92241	RCSD domain containing 1
SC326	18	KATNAL2	NM_031303	+	1	82	1	83473	katanin p60 subunit A-like 2
SC327	19	CYTH2	NM_004228;NM_017457	+	1	11271;11271	1	9266	cytohesin 2
SC328	9	MAX.chr9.139159296-139159348	-	-	0	-	1	-	-
SC329	5	CCNI2	NM_001039780	+	1	43	1	645121	cyclin I family, member 2
SC330	21	MIR155HG	NR_001458	+	1	18	1	114614	MIR155 host gene (non-protein coding)
SC331	6	AIM1	NM_001624	+	1	1101	1	202	absent in melanoma 1
SC332	7	MAX.chr7.156701663-156701795	-	-	0	-	0	-	-
SC333	1	TAL1	NM_003189	-	0	-2345	1	6886	T-cell acute lymphocytic leukemia 1
SC334	5	MAX.chr5.77140806-77140873	-	-	0	-	1	-	-
SC335	15	MAX.chr15.28352323-28352494	-	-	0	-	1	-	-
SC336	6	MAX.chr6.29521540-29521681	-	-	0	-	1	-	-
SC337	14	RIN3	NM_024832	+	0	513	1	79890	Ras and Rab interactor 3
SC338	9	PLIN2	NM_001122	-	0	-208	1	123	perilipin 2
SC339	1	KLHL21	NM_014851	-	0	-568	1	9903	kelch-like 21 (Drosophila)
SC340	1	C1orf86	NM_001146310	-	0	2213	1	199990	chromosome 1 open reading frame 86
SC341	21	CLIC6	NM_053277	+	1	343	1	54102	chloride intracellular channel 6
SC342	3	CLDN11	NM_001185056;NM_005602	+	0	-1656;719	1	5010	claudin 11
SC343	6	SNX9	NM_016224	+	0	-351	1	51429	sorting nexin 9
SC344	12	MAX.chr12.125534296-125534343	-	-	0	-	1	-	-
SC345	12	CACNA2D4	NM_172364	-	0	121809	1	93589	calcium channel, voltage-dependent, alpha 2/delta subunit 4
SC346	10	MAX.chr10.119312919-119312997	-	-	0	-	1	-	-
SC347	13	MAX.chr13.25321012-25321114	-	-	0	-	1	-	-
SC348	9	NFIL3	NM_005384	-	0	2471	1	4783	nuclear factor, interleukin 3 regulated

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC349	4	6660582	6660692	1%	2%	13%	21%	28%	11%	23%	2.18	11.76	18.47	24.32	9.50	20.20
SC350	5	77140667	77140774	2%	2%	10%	15%	35%	9%	24%	1.33	5.81	8.99	20.78	5.24	14.34
SC351	5	42952165	42952265	1%	4%	12%	17%	28%	18%	30%	5.58	19.39	27.28	43.92	28.58	47.30
SC352	3	194118747	194118919	0%	2%	13%	26%	43%	19%	41%	6.97	38.31	74.49	125.02	55.43	117.27
SC353	1	2989976	2990065	1%	3%	4%	18%	32%	8%	9%	3.14	3.74	16.20	29.59	7.08	8.48
SC354	17	41363552	41363612	0%	0%	0%	2%	21%	6%	15%	0.76	0.60	4.16	55.55	15.12	39.77
SC355	10	77168201	77168360	1%	2%	3%	7%	29%	5%	9%	1.99	2.99	7.70	29.70	5.27	8.77
SC356	11	129243348	129243401	2%	5%	17%	29%	49%	41%	66%	2.48	8.04	13.91	22.93	19.19	31.01
SC357	10	119296757	119296802	1%	1%	1%	9%	28%	3%	6%	1.03	1.36	10.19	32.99	3.39	6.47
SC358	18	21199523	21199632	0%	1%	10%	8%	17%	5%	6%	4.06	33.90	26.89	56.35	15.34	21.73
SC359	1	8277482	8277571	1%	2%	10%	22%	48%	31%	59%	1.59	9.26	20.67	45.68	29.57	56.50
SC360	1	2990151	2990207	1%	3%	5%	18%	32%	10%	16%	3.23	4.95	19.48	34.02	10.98	17.06
SC361	1	44031599	44031658	0%	2%	24%	18%	21%	21%	18%	4.80	75.23	56.94	66.66	64.67	56.57
SC362	7	27232734	27232792	2%	5%	12%	21%	46%	20%	34%	2.43	6.01	10.74	23.94	10.31	18.04
SC363	14	77591546	77591607	0%	1%	1%	2%	17%	10%	2%	3.66	4.59	6.87	64.89	37.49	7.51
SC364	5	42952414	42952472	2%	5%	20%	25%	43%	35%	57%	2.78	10.31	13.24	22.51	17.99	29.67
SC365	15	41787637	41787674	0%	4%	14%	13%	43%	18%	3%	12.04	39.44	36.80	121.85	50.77	9.60
SC366	13	33924448	33924560	1%	2%	4%	12%	37%	5%	11%	1.75	2.62	8.62	26.18	3.24	7.69
SC367	1	203598589	203598624	1%	1%	2%	2%	13%	2%	2%	1.20	3.53	3.87	24.55	3.76	3.75
SC368	19	50393424	50393465	1%	3%	11%	4%	25%	6%	40%	4.96	18.14	6.73	42.96	10.89	68.93
SC369	12	125534190	125534241	0%	1%	1%	8%	24%	5%	0%	4.31	5.32	46.29	134.03	25.97	0.00
SC370	15	41787438	41787549	1%	4%	19%	11%	45%	16%	2%	3.98	19.46	11.22	47.23	16.68	1.89
SC371	15	41787592	41787634	1%	6%	15%	13%	46%	18%	3%	5.95	15.44	13.55	46.26	18.60	3.53
SC372	8	38323572	38323653	0%	1%	4%	1%	10%	6%	6%	2.27	17.67	2.23	46.12	26.23	27.48

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC349	4	MAX.chr4.6660582-6660692	-	-	0	-	1	-	-
SC350	5	MAX.chr5.77140667-77140774	-	-	0	-	1	-	-
SC351	5	MAX.chr5.42952165-42952265	-	-	0	-	1	-	-
SC352	3	GP5	NM_004488	-	1	1248	1	2814	glycoprotein V (platelet)
SC353	1	PRDM16	NM_199454;NM_022114	+	0	4235;4235	0	63976	PR domain containing 16
SC354	17	NBR1	NM_031858;NM_031862;NM_005899	+	1	41042;41055;40307	0	4077	neighbor of BRCA1 gene 1
SC355	10	NCRNA00245	NR_024421;NR_024422	+	1	6916;4688	1	100131213	non-protein coding RNA 245
SC356	11	BARX2	NM_003658	+	0	-2532	1	8538	BARX homeobox 2
SC357	10	EMX2OS	NR_002791	-	0	7822	1	196047	EMX2 opposite strand (non-protein coding)
SC358	18	ANKRD29	NM_173505	-	1	43326	1	147463	ankyrin repeat domain 29
SC359	1	MAX.chr1.8277482-8277571	-	-	0	-	1	-	-
SC360	1	PRDM16	NM_199454;NM_022114	+	0	4410;4410	1	63976	PR domain containing 16
SC361	1	PTPRF	NM_002840;NM_130440	+	0	35053;35053	1	5792	protein tyrosine phosphatase, receptor type, F
SC362	7	MAX.chr7.27232734-27232792	-	-	0	-	1	-	-
SC363	14	MAX.chr14.77591546-77591607	-	-	0	-	1	-	-
SC364	5	MAX.chr5.42952414-42952472	-	-	0	-	0	-	-
SC365	15	ITPKA	NM_002220	+	0	1516	1	3706	inositol 1,4,5-trisphosphate 3-kinase A
SC366	13	MAX.chr13.33924448-33924560	-	-	0	-	1	-	-
SC367	1	ATP2B4	NM_001001396;NM_001684	+	0	2662;2662	1	493	ATPase, Ca++ transporting, plasma membrane 4
SC368	19	IL4I1	NM_152899;NM_172374	-	1	6723;39338	1	259307	interleukin 4 induced 1
SC369	12	MAX.chr12.125534190-125534241	-	-	0	-	1	-	-
SC370	15	ITPKA	NM_002220	+	0	1317	1	3706	inositol 1,4,5-trisphosphate 3-kinase A
SC371	15	ITPKA	NM_002220	+	0	1471	1	3706	inositol 1,4,5-trisphosphate 3-kinase A
SC372	8	FGFR1	NM_001174064;NM_023105;NM_001174067;NM_015850;NM_001174063;NM_023110;NM_001174065;NM_001174066;NM_023106	-	0	2780;2780;1791;2780;2780;2780;1791;1791;2780	0	2260	fibroblast growth factor receptor 1

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SC373	22	42470432	42470495	0%	1%	2%	1%	11%	6%	1%	1.87	4.62	2.75	31.95	19.18	2.56
SC374	2	74782096	74782223	0%	1%	1%	10%	15%	22%	12%	2.00	2.91	35.72	55.09	79.79	42.48
SC375	1	203598746	203598782	0%	0%	2%	3%	17%	2%	3%	1.78	8.34	14.03	90.60	9.86	16.53
SC376	3	4910281	4910325	0%	0%	2%	3%	18%	1%	14%	0.71	7.43	11.02	62.84	2.23	50.53
SC377	14	24780133	24780195	1%	2%	12%	9%	29%	11%	2%	1.26	8.92	6.65	21.77	8.09	1.18
SC378	7	27196035	27196154	1%	3%	21%	31%	37%	18%	56%	3.72	24.32	35.89	42.10	20.22	64.49
SC379	1	6480815	6480859	1%	3%	11%	8%	40%	5%	11%	4.03	17.19	12.38	61.31	7.48	17.22
SC380	7	27195748	27195829	1%	5%	25%	36%	38%	24%	69%	3.68	17.21	25.20	26.39	16.93	48.45
SC381	5	178957576	178957695	1%	2%	15%	18%	41%	17%	24%	3.97	29.20	34.94	78.81	31.81	45.24
SC382	22	28198164	28198199	0%	2%	6%	7%	17%	2%	18%	3.07	11.31	13.16	33.78	4.83	36.56
SC383	5	77268624	77268718	1%	2%	12%	22%	34%	7%	21%	3.72	21.82	40.13	61.10	12.77	38.23
SC384	1	29586455	29586550	0%	5%	30%	21%	39%	7%	32%	14.94	93.26	66.91	121.84	22.87	101.64
SC385	4	13524253	13524378	1%	2%	7%	21%	28%	11%	23%	3.50	10.37	32.37	43.87	17.49	36.14
SC386	20	3052753	3052851	1%	3%	17%	19%	35%	11%	15%	5.20	29.24	32.93	60.92	19.81	26.66
SC387	1	78511734	78511827	1%	2%	15%	32%	24%	15%	51%	2.91	18.47	39.63	29.28	18.15	62.64
SC388	14	62217806	62217860	0%	1%	1%	15%	25%	2%	2%	4.07	2.61	62.74	103.40	7.25	7.45
SC389	1	232941214	232941254	0%	0%	2%	11%	10%	1%	1%	1.43	5.41	34.37	33.30	4.75	4.09
SC390	13	21649754	21649843	1%	2%	3%	10%	21%	5%	9%	2.62	3.88	12.11	24.61	5.85	10.94
SC391	18	5890808	5890841	1%	2%	1%	9%	35%	4%	16%	1.59	0.97	8.79	34.81	3.53	15.72
SC392	4	1161145	1161177	0%	1%	3%	1%	13%	7%	2%	4.72	13.21	6.11	53.63	28.35	7.75
SC393	5	134879621	134879709	1%	2%	5%	21%	40%	6%	35%	3.43	8.06	33.63	65.55	9.28	56.82
SC394	1	221052431	221052479	1%	2%	10%	17%	20%	2%	40%	3.56	15.30	26.04	30.31	2.76	60.01
SC395	2	232527285	232527325	1%	3%	4%	5%	32%	9%	17%	3.87	5.99	6.44	43.13	11.79	23.75

FIG. 4 (cont'd)

Small Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SC373	22	FAM109B	NM_001002034	+	0	178	1	150368	family with sequence similarity 109, member B
SC374	2	DOK1	NM_001197260;NM_001381	+	0	5950;585	1	1796	docking protein 1, 62kDa (downstream of tyrosine kinase 1)
SC375	1	ATP2B4	NM_001001396;NM_001684	+	0	2819;2819	1	493	ATPase, Ca++ transporting, plasma membrane 4
SC376	3	MAX.chr3.4910281-4910325	-	-	0	-	1	-	-
SC377	14	CIDEB	NM_014430	-	1	443	1	27141	cell death-inducing DFFA-like effector b
SC378	7	HOXA7	NM_006896	-	1	261	1	3204	homeobox A7
SC379	1	ESPN	NM_031475	+	0	-4032	1	83715	espin
SC380	7	HOXA7	NM_006896	-	0	548	1	3204	homeobox A7
SC381	5	MAX.chr5.178957576-178957695	-	-	0	-	1	-	-
SC382	22	MN1	NM_002430	-	0	-678	1	4330	meningioma (disrupted in balanced translocation) 1
SC383	5	MAX.chr5.77268624-77268718	-	-	0	-	1	-	-
SC384	1	PTPRU	NM_133178;NM_133177;NM_001195001;NM_005704	+	1	23428;23428;23428;23428	1	10076	protein tyrosine phosphatase, receptor type, U
SC385	4	MAX.chr4.13524253-13524378	-	-	0	-	1	-	-
SC386	20	OXT	NM_000915	+	1	488	1	5020	oxytocin, prepropeptide
SC387	1	GIPC2	NM_017655	+	1	146	1	54810	GIPC PDZ domain containing family, member 2
SC388	14	MAX.chr14.62217806-62217860	-	-	0	-	1	-	-
SC389	1	KIAA1383	NM_019090	+	1	577	1	54627	KIAA1383
SC390	13	MAX.chr13.21649754-21649843	-	-	0	-	0	-	-
SC391	18	TMEM200C	NM_001080209	-	1	1295	1	645369	transmembrane protein 200C
SC392	4	SPON2	NM_012445;NM_001199021;NM_001128325	-	1	5512;41605;5854	1	10417	spondin 2, extracellular matrix protein
SC393	5	MAX.chr5.134879621-134879709	-	-	0	-	1	-	-
SC394	1	HLX	NM_021958	+	0	-311	1	3142	H2.0-like homeobox
SC395	2	MAX.chr2.232527285-232527325	-	-	0	-	1	-	-

FIG. 5

Squamous Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SQ1	17	38347792	38347942	0%	0%	0%	6%	16%	14%	1%	1.33	0.67	29.58	87.04	72.74	6.92
SQ2	19	58951416	58951527	1%	1%	11%	14%	16%	17%	16%	0.66	13.68	16.97	19.94	21.00	20.20
SQ3	11	14926627	14926716	1%	1%	5%	16%	62%	16%	11%	1.78	10.57	30.66	119.53	31.77	21.41
SQ4	18	44526868	44526957	1%	1%	6%	5%	22%	18%	7%	1.31	10.36	9.07	36.70	29.96	12.10
SQ5	22	50987219	50987295	1%	3%	32%	35%	62%	36%	28%	2.08	21.52	23.39	41.49	23.87	18.76
SQ6	3	124860573	124860665	0%	1%	12%	34%	12%	37%	39%	1.55	29.73	84.15	28.68	91.45	97.00
SQ7	17	42287927	42287988	0%	0%	11%	15%	26%	18%	32%	1.27	71.79	95.09	167.27	117.62	206.97
SQ8	3	124860704	124860798	0%	1%	10%	21%	9%	35%	22%	1.98	23.37	51.32	20.78	84.00	51.80
SQ9	8	145106353	145106439	0%	2%	24%	29%	9%	25%	26%	4.89	59.66	71.50	22.47	62.77	65.19
SQ10	2	97193509	97193639	1%	1%	14%	7%	16%	13%	16%	2.04	26.57	14.00	30.21	23.54	29.84
SQ11	8	99960542	99960654	1%	3%	10%	20%	44%	29%	24%	2.42	9.58	18.79	40.90	26.85	22.50
SQ12	5	42995477	42995528	0%	5%	20%	35%	60%	39%	62%	18.40	82.13	141.62	245.53	158.96	254.67
SQ13	1	44031599	44031658	0%	2%	24%	18%	21%	21%	18%	4.80	75.23	56.94	66.66	64.67	56.57
SQ14	11	14926795	14926853	0%	2%	9%	26%	78%	27%	10%	6.70	27.57	84.20	249.90	86.19	33.11
SQ15	1	8277482	8277571	1%	2%	10%	22%	48%	31%	59%	1.59	9.26	20.67	45.68	29.57	56.50
SQ16	5	37834845	37834910	2%	5%	13%	29%	50%	39%	33%	3.22	8.38	18.64	32.37	25.14	21.61
SQ17	2	73147720	73147790	1%	3%	24%	36%	50%	28%	59%	3.25	26.10	38.89	54.76	30.96	64.51
SQ18	5	42995328	42995393	0%	4%	12%	28%	50%	30%	55%	24.55	77.50	185.42	325.38	198.95	362.13
SQ19	8	124173236	124173386	0%	1%	11%	15%	49%	14%	29%	7.50	63.79	86.89	276.90	78.49	166.63
SQ20	11	14926886	14926955	0%	3%	6%	29%	78%	21%	20%	8.70	18.97	99.05	266.28	70.91	68.97
SQ21	2	26407567	26407639	1%	1%	5%	12%	62%	27%	16%	1.73	9.78	21.33	112.60	49.01	28.67
SQ22	5	42995102	42995171	0%	4%	8%	21%	43%	32%	59%	37.37	88.00	220.17	459.69	335.17	627.38
SQ23	6	28303447	28303515	0%	0%	12%	1%	9%	18%	14%	5.88	197.58	14.33	149.96	301.34	228.62

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SQ1	17	RAPGEFL1	NM_016339	+	0	13551	0	51195	Rap guanine nucleotide exchange factor (GEF)-like 1
SQ2	19	ZNF132	NM_003433	-	1	173	1	7691	zinc finger protein 132
SQ3	11	MAX.chr11.14926627-14926716	-	-	0	-	1	-	-
SQ4	18	KATNAL2	NM_031303	+	1	82	1	83473	katanin p60 subunit A-like 2
SQ5	22	KLHDC7B	NM_138433	+	1	758	1	113730	kelch domain containing 7B
SQ6	3	SLC12A8	NM_001195483;NM_024628	-	0	69670;71036	1	84561	solute carrier family 12 (potassium/chloride transporters), member 8
SQ7	17	UBTF	NM_001076684;NM_001076683;NM_014233	-	0	8997;10323;7737	1	7343	upstream binding transcription factor, RNA polymerase I
SQ8	3	SLC12A8	NM_001195483;NM_024628	-	0	69539;70905	1	84561	solute carrier family 12 (potassium/chloride transporters), member 8
SQ9	8	OPLAH	NM_017570	-	1	9231	1	26873	5-oxoprolinase (ATP-hydrolysing)
SQ10	2	MAX.chr2.97193509-97193639	-	-	0	-	0	-	-
SQ11	8	OSR2	NM_001142462;NM_053001	+	0	3912;3912	1	116039	odd-skipped related 2 (Drosophila)
SQ12	5	MAX.chr5.42995477-42995528	-	-	0	-	0	-	-
SQ13	1	PTPRF	NM_002840;NM_130440	+	0	35053;35053	1	5792	protein tyrosine phosphatase, receptor type, F
SQ14	11	MAX.chr11.14926795-14926853	-	-	0	-	1	-	-
SQ15	1	MAX.chr1.8277482-8277571	-	-	0	-	1	-	-
SQ16	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	1	1084;1084;4937;748	1	2668	glial cell derived neurotrophic factor
SQ17	2	EMX1	NM_004097	+	0	3117	1	2016	empty spiracles homeobox 1
SQ18	5	MAX.chr5.42995328-42995393	-	-	0	-	1	-	-
SQ19	8	MAX.chr8.124173236-124173386	-	-	0	-	1	-	-
SQ20	11	MAX.chr11.14926886-14926955	-	-	0	-	1	-	-
SQ21	2	FAM59B	NM_001191033;NM_001168241	+	1	3983;11608	1	150946	family with sequence similarity 59, member B
SQ22	5	MAX.chr5.42995102-42995171	-	-	0	-	0	-	-
SQ23	6	ZNF323	NM_145909;NR_024164;NM_001135216;NM_030899;NM_001135215;NR_024165	-	0	18525;464;464;705;20601;705	0	64288	zinc finger protein 323

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SQ24	19	37095829	37095999	1%	1%	23%	23%	19%	19%	41%	1.82	28.94	28.12	23.98	23.78	50.92
SQ25	8	145106742	145106827	0%	2%	26%	34%	10%	21%	14%	5.40	81.76	108.02	31.02	68.54	45.26
SQ26	5	37834716	37834762	2%	4%	16%	25%	51%	36%	36%	2.51	9.42	14.77	30.36	21.39	21.50
SQ27	2	99439270	99439356	1%	3%	24%	31%	81%	32%	56%	2.54	19.21	25.16	66.32	26.30	45.23
SQ28	1	248020671	248020722	1%	4%	31%	40%	63%	33%	63%	3.31	23.46	30.04	47.26	24.66	47.74
SQ29	17	38348024	38348072	0%	0%	1%	7%	7%	15%	0%	0.84	2.22	14.28	13.90	29.67	0.00
SQ30	20	61560692	61560749	0%	1%	6%	13%	62%	19%	28%	5.65	24.19	56.55	258.74	79.08	115.84
SQ31	3	122296709	122296828	0%	3%	17%	23%	65%	25%	61%	5.87	39.97	54.78	151.39	57.49	143.28
SQ32	2	26407721	26407876	0%	1%	2%	11%	47%	18%	16%	2.39	8.36	36.01	160.15	60.97	56.07
SQ33	10	22541891	22541996	0%	2%	13%	22%	49%	18%	24%	5.71	36.56	63.36	141.74	52.16	69.90
SQ34	8	145105570	145105675	1%	3%	30%	26%	9%	29%	23%	3.15	29.77	25.41	9.23	28.54	22.59
SQ35	15	65116396	65116440	0%	1%	11%	21%	5%	25%	2%	5.61	51.82	98.18	22.13	114.94	10.41
SQ36	2	74782325	74782452	0%	2%	1%	10%	12%	21%	12%	20.45	11.21	82.60	106.15	183.80	103.64
SQ37	5	37834916	37835022	1%	5%	9%	28%	55%	37%	42%	4.35	7.78	25.11	49.26	33.19	37.95
SQ38	2	74782096	74782223	0%	1%	1%	10%	15%	22%	12%	2.00	2.91	35.72	55.09	79.79	42.48
SQ39	17	75370492	75370581	0%	1%	2%	8%	2%	10%	12%	1.80	4.40	17.44	3.81	22.86	26.56
SQ40	17	27940477	27940568	0%	1%	5%	9%	59%	22%	25%	3.44	21.98	34.20	238.86	87.69	99.96
SQ41	6	6004298	6004338	1%	5%	21%	40%	58%	34%	61%	7.31	33.74	64.23	93.83	54.50	99.42
SQ42	17	8054628	8054698	0%	1%	12%	5%	7%	12%	11%	16.75	186.95	78.17	101.84	186.95	166.28
SQ43	17	73073700	73073810	0%	0%	0%	15%	8%	17%	0%	1.26	1.07	39.05	21.72	46.29	0.42

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SQ24	19	ZNF382	NM_032825	+	0	-391	1	84911	zinc finger protein 382
SQ25	8	OPLAH	NM_017570	-	0	8842	1	26873	5-oxoprolinase (ATP-hydrolysing)
SQ26	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	0	1213;1213;5066;877	1	2668	glial cell derived neurotrophic factor
SQ27	2	C2orf55	NM_207362	-	1	113414	1	343990	chromosome 2 open reading frame 55
SQ28	1	TRIM58	NM_015431	+	1	171	1	25893	tripartite motif-containing 58
SQ29	17	RAPGEFL1	NM_016339	+	0	13783	0	51195	Rap guanine nucleotide exchange factor (GEF)-like 1
SQ30	20	DIDO1	NM_033081;NM_001193369;NM_022105;NM_080797;NM_001193370;NM_080796	-	0	8612;-2789;8612;8612;-2789;-2789	1	11083	death inducer-obliterator 1
SQ31	3	PARP15	NM_001113523	+	0	261	1	165631	poly (ADP-ribose) polymerase family, member 15
SQ32	2	FAM59B	NM_001191033;NM_001168241	+	1	4137;11762	1	150946	family with sequence similarity 59, member B
SQ33	10	MAX.chr10.22541891-22541996	-	-	0	-	1	-	-
SQ34	8	MAX.chr8.145105570-145105675	-	-	0	-	1	-	-
SQ35	15	PIF1	NM_025049	-	1	1442	1	80119	PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae)
SQ36	2	DOK1	NM_001197260;NM_001381	+	1	6179;814	1	1796	docking protein 1, 62kDa (downstream of tyrosine kinase 1)
SQ37	5	GDNF	NM_001190469;NM_001190468;NM_000514;NM_199231	-	1	1013;1013;4866;677	1	2668	glial cell derived neurotrophic factor
SQ38	2	DOK1	NM_001197260;NM_001381	+	0	5950;585	1	1796	docking protein 1, 62kDa (downstream of tyrosine kinase 1)
SQ39	17	SEPT9	NM_006640;NM_001113494;NM_001113492;NM_001113493;NM_001113491	+	0	54896;-1672;86520;1221;93001	1	10801	septin 9
SQ40	17	ANKRD13B	NM_152345	+	1	19951	1	124930	ankyrin repeat domain 13B
SQ41	6	NRN1	NM_016588	-	0	3335	1	51299	neuritin 1
SQ42	17	PER1	NM_002616	-	0	1125	1	5187	period homolog 1 (Drosophila)
SQ43	17	MAX.chr17.73073700-73073810	-	-	0	-	1	-	-

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SQ44	5	125930731	125930884	1%	1%	2%	2%	4%	16%	1%	1.35	2.90	2.71	5.70	25.34	2.38
SQ45	15	65116474	65116558	1%	1%	9%	15%	9%	18%	1%	1.58	12.74	21.62	13.62	26.30	1.26
SQ46	17	58499109	58499183	0%	1%	1%	5%	35%	18%	8%	4.22	4.26	17.93	124.48	63.79	28.58
SQ47	2	97193166	97193253	0%	1%	13%	8%	18%	12%	21%	1.71	42.20	25.79	55.43	37.48	67.24
SQ48	2	118981859	118981945	1%	3%	14%	33%	42%	28%	29%	4.14	18.40	42.45	53.71	35.86	36.89
SQ49	7	44349487	44349591	1%	2%	5%	12%	28%	22%	8%	2.50	6.77	18.13	42.68	32.86	12.23
SQ50	17	17627469	17627534	1%	1%	17%	7%	0%	19%	11%	0.77	21.31	8.72	0.57	23.63	14.12
SQ51	3	194208259	194208403	0%	1%	2%	5%	17%	19%	0%	13.82	28.44	67.56	241.94	276.52	0.90
SQ52	1	223936868	223936997	0%	1%	7%	18%	45%	16%	28%	2.13	26.29	63.20	158.81	57.13	99.50
SQ53	19	58238816	58238942	0%	1%	8%	15%	24%	17%	33%	8.09	94.63	175.66	277.57	203.93	390.90
SQ54	12	25055873	25055997	0%	1%	10%	22%	29%	12%	40%	4.32	36.00	77.51	103.02	44.17	140.87
SQ55	11	68622903	68622965	0%	1%	2%	9%	3%	18%	9%	3.04	8.34	32.82	11.85	69.07	34.24
SQ56	5	32710286	32710370	0%	1%	2%	7%	3%	12%	8%	1.27	5.55	15.98	7.31	29.70	18.40
SQ57	1	46632701	46632852	1%	1%	5%	11%	11%	13%	1%	2.20	7.44	18.33	18.34	20.29	1.03
SQ58	3	32443052	32443156	0%	0%	1%	8%	2%	10%	1%	1.22	1.50	20.54	5.05	26.69	2.69
SQ59	15	90319850	90319883	0%	0%	0%	4%	0%	13%	0%	0.84	0.00	10.45	0.72	34.01	0.00
SQ60	17	43339264	43339345	1%	1%	4%	11%	41%	12%	11%	1.45	8.50	22.19	82.48	23.48	21.17
SQ61	19	37288426	37288510	0%	1%	15%	22%	30%	19%	27%	1.79	39.22	55.94	78.85	49.81	69.01
SQ62	8	145104291	145104342	0%	1%	6%	13%	3%	12%	10%	3.80	26.23	54.77	13.90	49.84	41.94

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SQ44	5	ALDH7A1	NM_001182	-	1	351	1	501	aldehyde dehydrogenase 7 family, member A1
SQ45	15	PIF1	NM_025049	-	1	1364	1	80119	PIF1 5'-to-3' DNA helicase homolog (S. cerevisiae)
SQ46	17	C17orf64	NM_181707	+	0	-755	1	124773	chromosome 17 open reading frame 64
SQ47	2	MAX.chr2.97193166-97193253	-	-	0	-	1	-	-
SQ48	2	MAX.chr2.118981859-118981945	-	-	0	-	1	-	-
SQ49	7	CAMK2B	NM_172078;NM_172084;NM_172083;NM_001220;NM_172080;NM_172079;NM_172082;NM_172081	-	0	15743;15743;15743;15743;15743;15743;15743;15743	1	816	calcium/calmodulin-dependent protein kinase II beta
SQ50	17	RAI1	NM_030665	+	1	42683	1	10743	retinoic acid induced 1
SQ51	3	FLJ34208	NR_033929	+	1	391	1	401106	hypothetical LOC401106
SQ52	1	CAPN2	NM_001146068;NM_001748	+	0	47574;36750	1	824	calpain 2, (m/II) large subunit
SQ53	19	ZNF671	NM_024833	-	1	179	1	79891	zinc finger protein 671
SQ54	12	BCAT1	NM_001178092;NM_005504;NM_001178094;NM_001178091;NM_001178093	-	0	46520;46520;-551;46520;136	1	586	branched chain amino-acid transaminase 1, cytosolic
SQ55	11	MAX.chr11.68622903-68622965	-	-	0	-	1	-	-
SQ56	5	NPR3	NM_000908	+	0	-1378	1	4883	natriuretic peptide receptor C/guanylate cyclase C (atrionatriuretic peptide receptor C)
SQ57	1	MAX.chr1.46632701-46632852	-	-	0	-	1	-	-
SQ58	3	CMTM7	NM_138410;NM_181472	+	0	9890;9890	0	112616	CKLF-like MARVEL transmembrane domain containing 7
SQ59	15	MESP2	NM_001039958	+	1	262	1	145873	mesoderm posterior 2 homolog (mouse)
SQ60	17	C17orf46	NM_152343	-	0	215	1	124783	chromosome 17 open reading frame 46
SQ61	19	MAX.chr19.37288426-37288510	-	-	0	-	1	-	-
SQ62	8	MAX.chr8.145104291-145104342	-	-	0	-	1	-	-

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Start position	Stop position	mean BC.island	mean lung.normal. island	mean Adenocarcinoma Lung.island	mean Large cell Lung.island	mean Small cell Lung.island	mean Squamous Lung.island	mean undefined cancer Lung.island	Norm/BC	Ad/BC	LC/BC	SC/BC	SQ/BC	UND/BC
SQ63	8	145104247	145104276	0%	1%	5%	13%	3%	11%	7%	9.51	48.36	126.04	32.55	112.97	73.75
SQ64	3	128212061	128212125	0%	2%	10%	16%	4%	20%	25%	7.50	33.85	56.41	12.62	68.60	85.89
SQ65	21	38119920	38119971	0%	1%	0%	8%	7%	11%	9%	1.98	0.80	16.93	14.11	23.88	18.66
SQ66	19	37288523	37288615	1%	2%	19%	22%	26%	18%	30%	2.72	25.92	30.28	34.88	24.73	40.51
SQ67	1	226925087	226925208	0%	1%	3%	9%	2%	11%	1%	1.79	7.13	25.23	5.25	29.82	3.21
SQ68	19	17346575	17346695	0%	1%	9%	24%	2%	13%	20%	6.09	68.86	178.14	15.24	98.45	144.24

FIG. 5 (cont'd)

Squamous Cell marker region ref. #	Chromosome	Gene	Transcript	Strand	In Exon	Tss Distance	In CpG Island	Entrez ID	Gene title
SQ63	8	MAX.chr8.145104247-145104276	-	-	0	-	1	-	-
SQ64	3	GATA2	NM_032638;NM_001145661	-	0	-31;-4688	1	2624	GATA binding protein 2
SQ65	21	SIM2	NM_005069	+	0	47930	1	6493	single-minded homolog 2 (Drosophila)
SQ66	19	MAX.chr19.37288523-37288615	-	-	0	-	1	-	-
SQ67	1	ITPKB	NM_002221	-	1	1789	1	3707	inositol 1,4,5-trisphosphate 3-kinase B
SQ68	19	NR2F6	NM_005234	-	1	9576	1	2063	nuclear receptor subfamily 2, group F, member 6

FIG. 6

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:1	AGRN Target DNA	GTTCCCGGAACGGCCTCTTGGGGGCGTTCCAGCCCCACGGACCCGCAGGGAGTCCCCCGCCGCAATTTGCATGGGGC TCATTTGCATGACCCCGCCCCCGCGGGAGTCGGGGGCGC
SEQ ID NO:2	AGRN Converted DNA	GTTTTCGGAACGGTTTTTTTGGGGGCGTTTTAGTTTTACGGATTCGTAGGGAGTTTTCGTCGTAATTTGTATGGGGTTT ATTTGTATGATTTCGTTTCGCGCGGGAGTCGGGGGCGT
SEQ ID NO:3	AGRN Forward Primer	GGCGTTTTAGTTTTACGGATTCG
SEQ ID NO:4	AGRN Reverse Primer	ACAAATAAACCCCATACAAATTACGAC
SEQ ID NO:5	AGRN Flap oligonucleotide	CGCCGAGGCGAAAACTCCCT/3C6/
SEQ ID NO:6	ANGPT1 Target DNA	CGGATTCAACATGGGCAATGTGCCTACACTTTCATTCTTCCAGAACACGATGGCAACTGTCGTGAGAGTACGACAGA CCAGTACAACACAAACGCTCTGCAGAGAGATGCTCCACACGTGGAACCG
SEQ ID NO:7	ANGPT1 Converted DNA	CGGATTTAATATGGGTAATGTGTTTATATTTTTATTTTTTTAGAATACGATGGTAATTGTCGTGAGAGTACGATAGAT TAGTATAATATAAACGTTTTGTAGAGAGATGTTTTATACGTGGAATCG
SEQ ID NO:8	ANGPT1 Forward Primer	TTTTAGAATACGATGGTAATTGTCGT
SEQ ID NO:9	ANGPT1 Reverse Primer	ACATCTCTCTACAAAACGTTTATATTATACTAATC
SEQ ID NO:10	ANGPT1 Flap oligonucleotide	CGCCGAGGCTATCGTACTCT/3C6/
SEQ ID NO:11	ANKRD13B Target DNA	GGAGCTACGACGAGCAGCTGCGGCTGGCGATGGAACTGTCGGCGCAGGAGCAGGAGGAGAGGCGGCGGCGCGC GCGCCAGGAGGAGGAGGAGCTGGAGCGCATCCTGAG
SEQ ID NO:12	ANKRD13B Converted DNA	GGAGTTACGACGAGTAGTTGCGGTTGGCGATGGAATTGTCGGCGTAGGAGTAGGAGGAGAGGCGGCGGCGCGCG CGTTAGGAGGAGGAGGAGTTGGAGCGTATTTTGAG
SEQ ID NO:13	ANKRD13B Forward Primer	AGTTACGACGAGTAGTTGCG
SEQ ID NO:14	ANKRD13B Reverse Primer	TCCTCCTACTCCTACGCC
SEQ ID NO:15	ANKRD13B Flap oligonucleotide	CCACGGACGCGACAATTCCAT/3C6/
SEQ ID NO:16	ARHGEF4 Target DNA	GGTGGCAACGGCTGGAGTGCCGTCGCCCGCGCCACTCACCCGGCGCGGCGCCCTGCGCGGCCGCTCAGCGGAAG GCCAGCAGGAAGATCAGTACGACGTTGATGAGAACCAGGAGCGCCAGCACGGCGGAGACCACCACGCG
SEQ ID NO:17	ARHGEF4 Converted DNA	GGTGGTAACGGTTGGAGTGTCGTCGTTCGCGTTATTTATTTCGGCGGCGTTTTGCGCGGTCGTTTAGCGGAAGG TTAGTAGGAAGATTAGTACGACGTTGATGAGAATTAGGAGCGTTAGTACGGCGGAGATTATTACGCG
SEQ ID NO:18	ARHGEF4 Forward Primer	CGTTCGCGTTATTTATTTCGGCG
SEQ ID NO:19	ARHGEF4 Reverse Primer	GCTCCTAATTCTCATCAACGTCGT
SEQ ID NO:20	ARHGEF4 Flap oligonucleotide	CGCCGAGGGCGGCGTTTTGC/3C6/
SEQ ID NO:21	BARX1 Target DNA	GGCCCGGGGCCGCCTGGGCCCCTAGGGGCTGGACGTCAACCTGTTAGATAGAGGGCGTGGGACCCCCCCGCAGGCG GCTGCTCGGACGACCGCATCCGGAG
SEQ ID NO:22	BARX1 Converted DNA	GGTTCGGGGTCGTTTGGGTTTTTAGGGGTTGGACGTTAATTTGTTAGATAGAGGGCGTGGGATTTTTCGTAGGCGG TTGTTCGGACGATCGTATTCGGAG
SEQ ID NO:23	BARX1 Forward Primer	CGTTAATTTGTTAGATAGAGGGCG
SEQ ID NO:24	BARX1 Reverse Primer	ACGATCGTCCGAACAACC
SEQ ID NO:25	BARX1 Flap oligonucleotide	CCACGGACGCGCCTACGAAAA/3C6/
SEQ ID NO:26	BARX1 Reverse Primer Universal	TCCGAACAACCGCCTAC
SEQ ID NO:27	BARX1 Flap oligonucleotide Universal	CCACGGACGCGAAAAATCCCA/3C6/
SEQ ID NO:405	BARX1 Flap oligonucleotide v6	AGGCCACGGACGCGAAAAATCCCACGC/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:28	BCAT1 Target DNA	GCTTCCAGCCGCGCGCTCCGTGCCACTGCCGCTCTCTGCAGCCCCGCGTCCCCGCAGCCTCCCCATGGCCAGCCCGCT TCGCTCCGCTGCGGCCCTTGCCCGCCAGGTACCTCGAACCC
SEQ ID NO:29	BCAT1 Converted DNA	GTTTTTAGTCGCGCGTTTCGTGTTATTGTCGTTTTTTGTAGTTTCGCGTTTTCGTAGTTTTTTTATGGTTAGTTCGTTTC GTTTCGTTGCGGTTTTTGTTCGTTAGGTATTTCGAATTT
SEQ ID NO:30	BCAT1 Forward Primer	GTGTTATTGTCGTTTTTTGTAGTTTCG
SEQ ID NO:31	BCAT1 Reverse Primer	CGCAACGAAACGAAACGA
SEQ ID NO:32	BCAT1 Flap oligonucleotide	CGCCGAGGGCGTTTTCGTAG/3C6/
SEQ ID NO:33	BCL2L11 Target DNA	GCCCGCCGCACGCCGCAATGCTCCGCGCTCCCCGCGGGGTCGGGCGACTCAGACAGGGACCGGAAAAGAACCACG CAGAAGAAAGCCCTATTTCTTGTCGTCTGTTCCTGTGCAGCCTTGCAGCCTCGCCGCCCCCGCGT
SEQ ID NO:34	BCL2L11 Converted DNA	GTTCGTCGTACGTCGTAATGTTTCGCGTTTTTCGCGGGGTCGGGCGATTTAGATAGGGATCGGAAAAGAATTACGTA GAAGAAAGTTTTATTTTTTGTCGTTTGTTTTTGTGTAGTTTTGTAGTTTCGTCGTTTTCGCGT
SEQ ID NO:35	BCL2L11 Forward Primer	CGTAATGTTTCGCGTTTTTCG
SEQ ID NO:36	BCL2L11 Reverse Primer	ACTTTCTTCTACGTAATTCTTTTCCGA
SEQ ID NO:37	BCL2L11 Flap oligonucleotide	CGCCGAGGGCGGGGTCGGGC/3C6/
SEQ ID NO:38	BHLHE23 Target DNA	GCCGGGGAGTCGAGAAGCAAGTACTAGCGCTCCAGGACCGCGCGCGCCGCCCCGCGCCGCCCCGCGCCGCCCCTC GGTCCAGAGC
SEQ ID NO:39	BHLHE23 Converted DNA	GTCGGGGAGTCGAGAAGTAAGTATTAGCGTTTTAGGATCGCGCGCGTCGTTTCGCGTCGTTTCGCGTCGTTTTTCGG TTTAGAGT
SEQ ID NO:40	BHLHE23 Forward Primer	AGTATTAGCGTTTTAGGATCGCG
SEQ ID NO:41	BHLHE23 Reverse Primer	ACTCTAAACCGAAAAACGACG
SEQ ID NO:42	BHLHE23 Flap oligonucleotide	CCACGGACGGCGAAACGACGC/3C6/
SEQ ID NO:43	BIN2 Target DNA	GCCGGGAGCCCGCACTTCCTCCTCGGGGGCCTCAGAAAACCACAGGGCGCGGGGCCAGGGCGGCGGCCCC CAGG
SEQ ID NO:44	BIN2 Converted DNA	GTCGGGAGTTCGTATTTTTTTTTCGGGGGTTTTAGAAAATTATAGGGCGCGGGGTTAGGGCGGCGGTTTT TAGG
SEQ ID NO:45	BIN2 Forward Primer	TCGGGAGTTCGTATTTTTTTTTCGG
SEQ ID NO:46	BIN2 Reverse Primer	AAAACCGCCGCCCTAAC
SEQ ID NO:47	BIN2 Flap oligonucleotide	CGCCGAGGCCCCGCGCCCTA/3C6/
SEQ ID NO:48	BIN2_Z Target DNA	CGGGGCCTACCCTCAGGCAGCGCTCGCTCGAGGCCAGCTTCCGAGCTCCAACCCCTGCCCGAAACCTCGGCCTCACT G
SEQ ID NO:49	BIN2_Z Converted DNA	CGGGGTTTATTTTTAGGTAGCGTTCGTTCGAGGTTAGTTTTCGAGTTTTAATTTTTGTTCGAAATTTCGGTTTTATTG
SEQ ID NO:50	BIN2_Z Forward Primer	GGGTTTATTTTTAGGTAGCGTTCG
SEQ ID NO:51	BIN2_Z Reverse Primer	CGAAATTTCGAACAAAAATTAAAACTCGA
SEQ ID NO:52	BIN2_Z Flap oligonucleotide	CCACGGACGGTTCGAGGTTAG/3C6/
SEQ ID NO:53	CAPN2 Target DNA	TGTCCTGACACGATGGCCACAGGCACAGTTTGTGGTGATGCCCAGGGGCCCGCGCGGCCCCACGGTGGTCCAGTTT ACACTCGGGCCCCGCACTCCTGAAGTTCCGCGCGGGAGGAGAAGGGCGTCCCTTTCGCAGCTCGG

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:54	CAPN2 Converted DNA	TGTTTTGATACGATGGTTATAGGTATAGTTTGTGGTGATGTTTAGGGGTTCGCGCGGTTTTACGGTGGTTTAGTTTAT ATTCGGGTTTCGTATTTTTGAAGTTTCGCGCGGGAGGAGAAGGGCGTTTTTTTCGTAGTTCGG
SEQ ID NO:55	CAPN2 Forward Primer	TGATGTTTAGGGGTTCGCG
SEQ ID NO:56	CAPN2 Reverse Primer	CGAAACTTCAAAAATACGAAACCCGA
SEQ ID NO:57	CAPN2 Flap oligonucleotide	CGCCGAGG GCGGTTTTACGG/3C6/
SEQ ID NO:58	chr5_132 Target DNA	CCGGAGCACTCGCCGCTGCGCGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAGGCCGGCGGGCTGGGCGGCTC GGCAGCCTGCGCCGCGGCCTCCGCCTCGGCCGCCAGC
SEQ ID NO:59	chr5_132 Converted DNA	TCGGAGTATTCGTCGTTGCGCGTTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAGGTCGGCGGGTTGGGCGGTTCG GTAGTTTGCGTCGCGGTTTTCGTTTCGGTCGTTAGT
SEQ ID NO:60	chr5_132 Forward Primer	GTATTCGTCGTTGCGCG
SEQ ID NO:61	chr5_132 Reverse Primer	CCTCGAAAACCGCCTACC
SEQ ID NO:62	chr5_132 Flap oligonucleotide	CCACGGACGCGCCAACGACTT/3C6/
SEQ ID NO:63	chr7_636 Target DNA	CGCCGTGAGTGTTATAGTTCTTAAAGGCGGCGTGTCCGGAGTTTCTTCCTTCTGGTGGGGTTCGTGGTCTCGCCGGC TCAGGAGTGAAGCTGCAGATCTTCGCGGTGAGTGTTACAGCTCCTAAGGCGGCGCAT
SEQ ID NO:64	chr7_636 Converted DNA	CGTCGTGAGTGTTATAGTTTTTAAAGGCGGCGTGTTCGGAGTTTTTTTTTTTTGGTGGGGTTCGTGGTTTCGTCGGTT TAGGAGTGAAGTTGTAGATTTTCGCGGTGAGTGTTATAGTTTTTAAGGCGGCGTAT
SEQ ID NO:65	chr7_636 Forward Primer	TAAAGGCGGCGTGTTCG
SEQ ID NO:66	chr7_636 Reverse Primer	CAACTTCACTCCTAAACCGAC
SEQ ID NO:67	chr7_636 Flap oligonucleotide	CCACGGACGCGAAACCACGAA/3C6/
SEQ ID NO:68	CYP26C1 Target DNA	AACTGGCCTTCTGGCTACTCCGGAATCGCCAAGCAGATGAGGCCAGACCGCCGCCAGCGCTGATCACGCGCGCTCC CACAGGTCCTGGCGCGCGTGTTCAGCCGCGC
SEQ ID NO:69	CYP26C1 Converted DNA	AATTGGTTTTTTGGTTATTTCGGAATCGTTAAGTAGATGAGGTTAGATCGTCGTTAGCGTTGATTACGCGCGTTTTTA TAGGTTTTGGCGCGCGTGTTTAGTCGCGT
SEQ ID NO:70	CYP26C1 Forward Primer	TGGTTTTTTGGTTATTTCGGAATCGT
SEQ ID NO:71	CYP26C1 Reverse Primer	GCGCGTAATCAACGCTAAC
SEQ ID NO:72	CYP26C1 Flap oligonucleotide	CGCCGAGGCGACGATCTAAC/3C6/
SEQ ID NO:73	DIDO1 Target DNA	GGAGCGGGCAGAGGAGGAGCCCAGCGCCGAGGCCCAGGCGCGCCCCGCCCTCGCCCCTCCCCGTGCCCCTCCCCCG CTGCTCCCC
SEQ ID NO:74	DIDO1 Converted DNA	GGAGCGGGTAGAGGAGGAGTTTAGCGTCGAGGTTTAGGCGCGTTTCGTTTTCGTTTTTTTTCGTGTTTTTTTTTCGTT GTTTTTT
SEQ ID NO:75	DIDO1 Forward Primer	GAGGAGGAGTTTAGCGTCG
SEQ ID NO:76	DIDO1 Reverse Primer	CACGAAAAAAAAACGAAAACGAAAC
SEQ ID NO:77	DIDO1 Flap oligonucleotide	CGCCGAGGCGCGCCTAAACC/3C6/
SEQ ID NO:78	DLX4 Target DNA	GCGGTCTATCACGGGCACCCCTAACACTTGGTGAGTGCGCAGTGCTCTCGGCAGTCTCTGGGCTCCATACGATGCCT ACCGCACGCCCTAGCAGAGGAGGTCTCTGT
SEQ ID NO:79	DLX4 Converted DNA	GCGGTTTATTACGGGTATTTTTAATATTTGGTGAGTGCGTAGTGTTTTCGGTAGTTTTTGGGTTTTATACGATGTTTAT CGTACGTTTTAGTAGAGGAGGTTTTTGT
SEQ ID NO:80	DLX4 Forward Primer	TGAGTGCGTAGTGTTTTCGG
SEQ ID NO:81	DLX4 Reverse Primer	CTCCTCTACTAAAACGTACGATAAACA

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:82	DLX4 Flap oligonucleotide	CGCCGAGGATCGTATAAAAC/3C6/
SEQ ID NO:83	DLX4 Forward Primer Universal	ATATTTGGTGAGTGCGTAGTG
SEQ ID NO:84	DLX4 Reverse Primer Universal	ACGTACGATAAACATCGTATAAAACC
SEQ ID NO:85	DLX4 Flap oligonucleotide Universal	CGCCGAGGGTTTTCGGTAGT/3C6/
SEQ ID NO:86	DMRTA2 Target DNA	TACTCCACTGCCGGCTTGGTGCCCACGCTCGGCTTCCGCCCACCCATGGACTACGCCTTTAGCGATCTCATGCGTGAC CGCTCGGCCGCCGCTGCTGCGGCGGTGCACAAGGAGCCGACCT
SEQ ID NO:87	DMRTA2 Converted DNA	TATTTTATTGTCGGTTTGGTGTTTACGTTCGGTTTTCGTTTATTTATGGATTACGTTTTTAGCGATTTTATGCGTGATCG TTCGGTCGTCGTTGTTGCGGCGGTGTATAAGGAGTCGATTT
SEQ ID NO:88	DMRTA2 Forward Primer	TGGTGTTTACGTTCGGTTTTCGT
SEQ ID NO:89	DMRTA2 Reverse Primer	CCGCAACAACGACGACC
SEQ ID NO:90	DMRTA2 Flap oligonucleotide	CGCCGAGGCGAACGATCACG/3C6/
SEQ ID NO:91	DNMT3A Target DNA	AGGCCGGTCACGAACAAAGCGCTGGCGAGTGCGCGCCCGCCCACGCGCACAGGTGCCCGCGACAAGACGCCCCGT CCCCGCCCACGCGGCCCCCGCGGGCTGAGCC
SEQ ID NO:92	DNMT3A Converted DNA	AGGTCGGTTACGAATAAAGCGTTGGCGAGTGCGCGTTCGTTTACGCGTATAGGTGTTCGCGATAAGACGTTTCGTTT TCGTTTACGCGGTTTTCGCGGGTTGAGTT
SEQ ID NO:93	DNMT3A Forward Primer	GTTACGAATAAAGCGTTGGCG
SEQ ID NO:94	DNMT3A Reverse Primer	AACGAAACGTCTTATCGCGA
SEQ ID NO:95	DNMT3A Flap oligonucleotide	CCACGGACGGAGTGCGCGTTC/3C6/
SEQ ID NO:96	DOCK2 Target DNA	GCCGGCCCCGCAGCATCCTCCTGCTCGCGGCTCTCCCGCCACCTGTCCCGCTCCCTGCCGCGCCCTGGGGCCCGCACC TACCCAC
SEQ ID NO:97	DOCK2 Converted DNA	GTCGGTTTCGTAGTATTTTTTTGTTCGCGGTTTTTTCGTTATTTGTTTCGTTTTTTGTCGCGTTTTGGGGTTCGTATTTA TTTAT
SEQ ID NO:98	DOCK2 Forward Primer	CGGTTTCGTAGTATTTTTTTGTTCG
SEQ ID NO:99	DOCK2 Reverse Primer	GAACCCCAAAACGCGAC
SEQ ID NO:100	DOCK2 Flap oligonucleotide	CGCCGAGGGCGGTTTTTTCG/3C6/
SEQ ID NO:101	DTX1 Target DNA	CGCCTCCTGGGCTCCCCCCGGAGTGGGAGGGAGCCGCGGTCCCGCCTCCGCGCCCGTTCCCTCCCAGGCCCCTCGGC CGCCGCGCCGAGCTTTCCGCGCGTGGACAGACTGCCCGGCCGACGGACGGACGCAGG
SEQ ID NO:102	DTX1 Converted DNA	CGTTTTTTGGGTTTTTTTCGGAGTGGGAGGGAGTCGCGGTTTCGTTTTCGCGTTCGTTTTTTTTTAGGTTTTTCGGTCG TCGCGTCGAGTTTTTCGCGCGTGGATAGATTGTTCGGTCGACGGACGGACGTAGG
SEQ ID NO:103	DTX1 Forward Primer	GAGTCGCGGTTTCGTTTTC
SEQ ID NO:104	DTX1 Reverse Primer	GACGCGACGACCGAAAAAC
SEQ ID NO:105	DTX1 Flap oligonucleotide	CGCCGAGGCGCGTTCGTTTT/3C6/
SEQ ID NO:106	EMX1 Target DNA	TCCGGCGCCGCGTTTTCTAGAGAACCGGGTCTCAGCGATGCTCATTTCAGCCCCGTCTTAATGCAACAAACGAAACC CCACACGAACGAAAAGGAACATGTCTGCGCT
SEQ ID NO:107	EMX1 Converted DNA	TCGGCGTCGCGTTTTTTAGAGAATCGGGTTTTAGCGATGTTTATTTTAGTTTCGTTTTAATGTAATAAACGAAATTTTA TACGAACGAAAAGGAATATGTTTGCGTT
SEQ ID NO:108	EMX1 Forward Primer	GGCGTCGCGTTTTTTAGAGAA
SEQ ID NO:109	EMX1 Reverse Primer	TTCCTTTTCGTTCGTATAAAATTTCGTT
SEQ ID NO:110	EMX1 Flap oligonucleotide	CCACGGACGATCGGGTTTTAG/3C6/

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:111	FAM59B Target DNA	GGGCCTGCTGGCCGGGGACCCGCGCGTCGAGCGCCTGGTGCGCGACAGCGCCTCCTACTGCCGCGAGCGCTTCGAC CCCGACGAGTACTCCACGGCCGTGCGCGAGGCGCCAGCGGAGCTCGCCGAAG
SEQ ID NO:112	FAM59B Converted DNA	GGGTTTGTTGGTCGGGGATTCGCGCGTCGAGCGTTTGGTGCGCGATAGCGTTTTTTATTGTCGCGAGCGTTTCGATT TCGACGAGTATTTTACGGTCGTGCGCGAGGCGTTAGCGGAGTTCGTCGAAG
SEQ ID NO:113	FAM59B Forward Primer	CGATAGCGTTTTTTATTGTCGCG
SEQ ID NO:114	FAM59B Reverse Primer	GCACGACCGTAAAATACTCGTC
SEQ ID NO:115	FAM59B Flap oligonucleotide	CCACGGACGCGAAATCGAAAC/3C6/
SEQ ID NO:406	FAM59B Forward Primer v3	GTCGAGCGTTTGGTGCG
SEQ ID NO:407	FAM59B Reverse Primer v3	CTCGTCGAAATCGAAACGC
SEQ ID NO:408	FAM59B Flap oligonucleotide v3	CGCGCCGAGGGCGATAGCGTTTTTTATTGTCG/3C6/
SEQ ID NO:116	FERMT3 Target DNA	TAGCAGCAGCCGCAGCCATGGCGGGGATGAAGACAGCCTCCGGGGACTACATCGACTCGTCATGGGAGCTGCGGG TGTTTGTGGGAGAGGAGGACCCAGAGGCCGAGTCGGTCACCCTGCGGGTCACTGGGGAGTCGCAC
SEQ ID NO:117	FERMT3 Converted DNA	TAGTAGTAGTCGTAGTTATGGCGGGGATGAAGATAGTTTTCGGGGATTATATCGATTCGTTATGGGAGTTGCGGGT GTTTGTGGGAGAGGAGGATTTAGAGGTCGAGTCGGTTATTTTGCGGGTTATTGGGGAGTCGTAT
SEQ ID NO:118	FERMT3 Forward Primer	GTTTTCGGGGATTATATCGATTCG
SEQ ID NO:119	FERMT3 Reverse Primer	CCCAATAACCCGCAAAATAACC
SEQ ID NO:120	FERMT3 Flap oligonucleotide	CGCCGAGGCGACTCGACCTC/3C6/
SEQ ID NO:121	FGF14 Target DNA	GTCCCAGAGACGCCCTAGGGTCAGAGGTCATCTCCGTGGCAACGGAAACTTCCCGCGCTACGGCGGCTCCAACGGG CCGCTTCCGCCGCATTGCGTAGCGAAGC
SEQ ID NO:122	FGF14 Converted DNA	GTTTTAGAGACGTTTTAGGGTTAGAGGTTATTTTCGTGGTAACGGAAATTTTTCGCGTTACGGCGGTTTTAACGGGT CGTTTTCGTCGTATTGCGTAGCGAAGT
SEQ ID NO:123	FGF14 Forward Primer	TTTCGTGGTAACGGAAATTTTTCG
SEQ ID NO:124	FGF14 Reverse Primer	CGACGAAAACGACCCGT
SEQ ID NO:125	FGF14 Flap oligonucleotide	CGCCGAGGGCGTTACGGCGG/3C6/
SEQ ID NO:126	FLJ34208 Target DNA	GCGCCCCGGCCGCAGGCGGAGGACAGGGAGGAGCGCACACGAGAAAGCTCCCACGCGCCCGCGCCTCGCCTCCGA CGGGAAGGCGCCTCTTCCGACCGTCCTGGATG
SEQ ID NO:127	FLJ34208 Converted DNA	GCGTTTCGGTCGTAGGCGGAGGATAGGGAGGAGCGTATACGAGAAAGTTTTTACGCGTTCGCGTTTCGTTTTCGAC GGGAAGGCGTTTTTTTCGATCGTTTTGGATG
SEQ ID NO:128	FLJ34208 Forward Primer	GAGCGTATACGAGAAAGTTTTTACG
SEQ ID NO:129	FLJ34208 Reverse Primer	AACGCCTTCCCGTCGAA
SEQ ID NO:130	FLJ34208 Flap oligonucleotide	CCACGGACG GCGTTCGCGTTT/3C6/
SEQ ID NO:131	FLJ45983 Target DNA	CGAGAGGGCGCGAGCACAGCCGAGGCCATGGAGGTGACGGCGGACCAGCCGCGCTGGGTGAGCCACCACCACCC CGCCGTGCTCAACGGGCAGCACCCGGACACGCAC
SEQ ID NO:132	FLJ45983 Converted DNA	CGAGAGGGCGCGAGTATAGTCGAGGTTATGGAGGTGACGGCGGATTAGTCGCGTTGGGTGAGTTATTATTATTTC GTCGTGTTTAACGGGTAGTATTCGGATACGTAT
SEQ ID NO:133	FLJ45983 Forward Primer	GGGCGCGAGTATAGTCG
SEQ ID NO:134	FLJ45983 Reverse Primer	CAACGCGACTAATCCGC
SEQ ID NO:135	FLJ45983 Flap oligonucleotide	CGCCGAGGCCGTCACCTCCA/3C6/

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:409	FLJ45983 Forward Primer v4	CGAGGTTATGGAGGTGACG
SEQ ID NO:410	FLJ45983 Reverse Primer v4	CGAATACTACCCGTTAAACACG
SEQ ID NO:411	FLJ45983_Flap oligonucleotide v4	AGGCCACGGACGGGCGGATTAGTCGCG/3C6/
SEQ ID NO:136	GRIN2D Target DNA	CGCCCCCTCACCTCCCCGATCATGCCGTTCCAGACGCCATCGATCTTCTTTCCGTGCTTGCCATTGGTGACCAGGTAG AGGTCGTAGCTGAAGCCGATGGTATGCGCCAGCCGCTTCAGAATGTCGATGCAGAAACCCTTG
SEQ ID NO:137	GRIN2D Converted DNA	CGTTTTTTTATTTTTTCGATTATGTCGTTTTAGACGTTATCGATTTTTTTTTTCGTGTTTGTTATTGGTGATTAGGTAGAG GTCGTAGTTGAAGTCGATGGTATGCGTTAGTCGTTTTAGAATGTCGATGTAGAAATTTTTG
SEQ ID NO:138	GRIN2D Forward Primer	TCGATTATGTCGTTTTAGACGTTATCG
SEQ ID NO:139	GRIN2D Reverse Primer	TCTACATCGACATTCTAAAACGACTAAC
SEQ ID NO:140	GRIN2D Flap oligonucleotide	CCACGGACGCGCATACCATCG/3C6/
SEQ ID NO:141	HIST1H2BE Target DNA	CGGCGAGGCTTCCCGCCTGGCGCATTACAACAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCGC CTGCTGCTTCCCGGGGA
SEQ ID NO:142	HIST1H2BE Converted DNA	CGGCGAGGTTTTTCGTTTGGCGTATTATAATAAGCGTTCGATTATTATTTTTAGGGAGATTTAGACGGTCGTGCGTTT GTTGTTTTTCGGGGA
SEQ ID NO:143	HIST1H2BE Forward Primer	TGGCGTATTATAATAAGCGTTCG
SEQ ID NO:144	HIST1H2BE Reverse Primer	AACAACAAACGCACGACC
SEQ ID NO:145	HIST1H2BE Flap oligonucleotide	CCACGGACGCGTCTAAATCTC/3C6/
SEQ ID NO:412	HOPX Target DNA	GGCGGCGCCGCGACCGCCTTCCTTCGCTGCGTCCCGCCCGCTCCACGCCTCGCTCACCGCCGCCGCTTCTCCCTGCCC CGCAGCGCGCAGGGACCATGTCGGCGGAGACCGCGAGCGGCCCCACAGAGGACCAGGTGGAAATCCTGGAGTAC AACTTCAACAAGGTCGACAAGCACCCGGATTCCACCACGCTGTGCCTCATCGCGGCCGAGGCAGGCCTTTCCGAGG AGGAGACCCAGGTGCGTCCCCACACGCGCCCAGCGCGCCCCGACCCCTGCCTGGGCTGAGCCTTCTCGCGGCTGGG CGGTCCTGTTCGTCCGCGCCTCCCGC
SEQ ID NO:413	HOPX Converted DNA	GGCGGCGTCGCGATCGTTTTTTTTCGTTGCGTTTCGTTCGTTTTACGTTTCGTTTATCGTCGTCGTTTTTTTTTGTTTCG TAGCGCGTAGGGATTATGTCGGCGGAGATCGCGAGCGGTTTTATAGAGGATTAGGTGGAAATTTTGGAGTATAATT TTAATAAGGTCGATAAGTATTCGGATTTTATTACGTTGTGTTTTATCGCGGTCGAGGTAGGTTTTTTCGAGGAGGAG ATTTAGGTGCGTTTTTATACGCGTTTAGCGCGTTTCGATTTTTGTTTGGGTTGAGTTTTTTCGCGGTTGGGCGGTTTTG TTCGTTCGCGTTTTTCGT
SEQ ID NO:414	HOPX 2236 Forward Primer	GCGTCGCGATCGTTTTTTTTCG
SEQ ID NO:415	HOPX 2236 Reverse Primer	AACGACGACGATAAACGAAACGTA
SEQ ID NO:416	HOPX 2236 Flap oligonucleotide	AGGCCACGGACGGTTGCGTTTCGTTCGTTT/3C6/
SEQ ID NO:417	HOPX 2149 Forward Primer	GTAGCGCGTAGGGATTATGTCG
SEQ ID NO:418	HOPX 2149 Reverse Primer	TTTCCACCTAATCCTCTATAAAACCGC
SEQ ID NO:419	HOPX 2149 Flap oligonucleotide	AGGCCACGGACG CTCGCGATCTCCGC/3C6/
SEQ ID NO:420	HOPX Forward Primer	ACGTTGTGTTTTATCGCGG
SEQ ID NO:421	HOPX Reverse Primer	CTAAACGCGTATAAAAACGCAC
SEQ ID NO:422	HOPX Flap oligonucleotide	AGGCCACGGACGGTCGAGGTAGGTTTTTTCGA
SEQ ID NO:146	HOXA9 Target DNA	GGGCGGGCCAGGCGCTGGGCACGGTGATGGCCACCACTGGGGCCCTGGGCAACTACTACGTGGACTCGTTCCTGC TGGGCGCCGACGCCGCGGATGAGCTG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:147	HOXA9 Converted DNA	GGGCGGGTTAGGCGTTGGGTACGGTGATGGTTATTATTGGGGTTTTGGGTAATTATTACGTGGATTCGTTTTTGTTG GGCGTCGACGTCGCGGATGAGTTG
SEQ ID NO:148	HOXA9 Forward Primer	TTGGGTAATTATTACGTGGATTCG
SEQ ID NO:149	HOXA9 Reverse Primer	ACTCATCCGCGACGTC
SEQ ID NO:150	HOXA9 Flap oligonucleotide	CCACGGACGCGACGCCCAACA/3C6/
SEQ ID NO:423	HOXA9 Reverse Primer v2	CAACTCATCCGCGACG
SEQ ID NO:424	HOXA9 Flap oligonucleotide v2	AGGCCACGGACGGTCGACGCCCAACAA/3C6/
SEQ ID NO:151	HOXB2 Target DNA	GGGCCATTGCCAGAAGACGTCTTCTCGGGGCGCCAGGATTCACCTTTCCTTCCCGACCTCAACTTCTTCGCGGCCGAC TCCTGTCTCCAGCTATC
SEQ ID NO:152	HOXB2 Converted DNA	GGGTTATTGTTAGAAGACGTTTTTTCGGGGCGTTAGGATTTATTTTTTTTTTTCGATTTTAATTTTTTCGCGGTCGATTT TTGTTTTTAGTTATT
SEQ ID NO:153	HOXB2 Forward Primer	GTTAGAAGACGTTTTTTCGGGG
SEQ ID NO:154	HOXB2 Reverse Primer	AAAACAAAAATCGACCGCGA
SEQ ID NO:155	HOXB2 Flap oligonucleotide	CGCCGAGGGCGTTAGGATTT/3C6/
SEQ ID NO:425	HOXB2 Flap oligonucleotide 2	CGCGCCGAGGGCGTTAGGATTTATTTTTTTTTTTCGA/3C6/
SEQ ID NO:426	IFFO1 Target DNA	CGGGACAGAGCCGACCAATCAGGCGGCTCGGCAGCGGGGCAGAGGTCAGGGGGCGGGCCGAGGGGAAGCCAA
SEQ ID NO:427	IFFO1 Converted DNA	CGGGATAGAGTCGATTAATTAGGCGGTTCGGTAGCGGGGTAGAGGTTAGGGGGCGGGTCGAGGGGAAGTTAA
SEQ ID NO:428	IFFO1 Forward Primer	CGGGATAGAGTCGATTAATTAGGC
SEQ ID NO:429	IFFO1 Reverse Primer	TAACTTCCCCTCGACCCG
SEQ ID NO:430	IFFO1 Flap oligonucleotide	CGCGCCGAGGCGGTTCGGTAGCGG/3C6/
SEQ ID NO:156	KLHDC7B Target DNA	GGCCCCGGAAGCCCAGCTCCCGGGCCCTGGAGCCCGCCACGGCGGCAGCCCTGCGGCGGCGGCTGGACCTGGGCA GTTGCCTGGACGTGCTGGCCTTTGCCCAGCA
SEQ ID NO:157	KLHDC7B Converted DNA	GGTTTCGGAAGTTTAGTTTTCGGGTTTTGGAGTTCGTTACGGCGGTAGTTTTGCGGCGGCGGTTGGATTTGGGTAGT TGTTTGGACGTGTTGGTTTTTGTTTAGTA
SEQ ID NO:158	KLHDC7B Forward Primer	AGTTTTCGGGTTTTGGAGTTCGTTA
SEQ ID NO:159	KLHDC7B Reverse Primer	CCAAATCCAACCGCCGC
SEQ ID NO:160	KLHDC7B Flap oligonucleotide	CGCCGAGGACGGCGGTAGTT/3C6/
SEQ ID NO:161	LOC100129726 Target DNA	GGCGGCGCCGGCGGCTGCGCGGGGGGGCGCCAGGCCCTGCTGCTGCTGCTGCTGCTGACTGCGGTAGTAGGCGGC GGCGGCCACGGCGGCAAAGTTGTGGGTCTGGA
SEQ ID NO:162	LOC100129726 Converted DNA	GGCGGCGTCGGCGGTTGCGCGGGGGGCGTTAGGTTTTGTTGTTGTTGTTGTTGTTGATTGCGGTAGTAGGCGGCG GCGGTTACGGCGGTAAAGTTGTGGGTTTGGA
SEQ ID NO:163	LOC100129726 Forward Primer	TTGATTGCGGTAGTAGGCG
SEQ ID NO:164	LOC100129726 Reverse Primer	AACCCACAACTTTACCGCC
SEQ ID NO:165	LOC100129726 Flap oligonucleotide	CGCCGAGGCGTAACCGCCGC/3C6/
SEQ ID NO:166	MATK Target DNA	GGTTTCCCCCCACCCCGGCCTCGGGGTCTCTCCACGTCTCCCCGCCGACGTGCTCACCTGCTCAGGGGGCGCCCCCGA GCCGCGCCCCGCGCCCGCCCCCAGGAGGGCCTCCGCGAGCCGGCTGCACACCCCGAGGCGGTCCCGGCTGCACAAC

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:167	MATK Converted DNA	GGTTTTTTTTTATTTCGGTTTCGGGGTTTTTTTACGTTTTTTCGTCGACGTGTTTATTTGTTTAGGGGGCGTTTTCGAGT CGCGTTTCGCGTTCGTTTTTAGGAGGGTTTTCGCGAGTCGGTTGTATATTTCGAGGCGGTTTCGGTTGTATAAT
SEQ ID NO:168	MATK Forward Primer	GTTTCGGGGTTTTTTTACGTTTTTTCG
SEQ ID NO:169	MATK Reverse Primer	AAACGCGACTCGAAAACGC
SEQ ID NO:170	MATK Flap oligonucleotide	CGCCGAGGGTCGACGTGTTT/3C6/
SEQ ID NO:171	MAX_Chr10.225 Target DNA	CTCCGGTTTTCGCGGTTCTCAGCGATATTAGGCGCGGCCAGTGTCTGAAAGCTCCTCGGGGTTACGTCCTGGGGCGA CTGGAGGCGGCTCACGAC
SEQ ID NO:172	MAX_Chr10.225 Converted DNA	TTTCGGTTTTCGCGGTTTTTAGCGATATTAGGCGCGGTTAGTGTTTGAAAGTTTTTCGGGGTTACGTTTTGGGGCGAT TGGAGGCGGTTTACGAT
SEQ ID NO:173	MAX_Chr10.225 Forward Primer	CGGTTTTTAGCGATATTAGGCG
SEQ ID NO:174	MAX_Chr10.225 Reverse Primer	CCCAAAACGTAACCCCGA
SEQ ID NO:175	MAX_Chr10.225 Flap oligonucleotide	CGCCGAGGGCGGTTAGTGTT/3C6/
SEQ ID NO:176	MAX_Chr10.226 Target DNA	CGACGGCCGCGGAGGAGGAAGGCCAGGGGGAAATTTGCATTTCGTAAAACCGCGGTTAAGAAATGACGATGCCAC GTAGACAAGCCAGTTGTGACGTTCAGCACAACGTGCTACTGAACTACCGAGATCCGCCACCAAATGGC
SEQ ID NO:177	MAX_Chr10.226 Converted DNA	CGACGGTCGCGGAGGAGGAAGGTTAGGGGGAAATTTGTATTTCGTAAAATCGCGGTTAAGAAATGACGATGTTAC GTAGATAAGTTAGTTGTGACGTTTAGTATAACGTGTTATTGAATTATCGAGATTCGTTATTAAATGGT
SEQ ID NO:178	MAX_Chr10.226 Forward Primer	GGGAAATTTGTATTTCGTAAAATCG
SEQ ID NO:179	MAX_Chr10.226 Reverse Primer	ACAACTAACTTATCTACGTAACATCGT
SEQ ID NO:180	MAX_Chr10.226 Flap oligonucleotide	CCACGGACGGCGGTTAAGAAA/3C6/
SEQ ID NO:181	MAX.chr12.52 Target DNA	GGCTTGGGGTCCAGCCGCCCGCCCCTGCCGCCACCGCACCATGTCCTGCCTCTACTCCCGCCTCAGCGCCCCCTGCGG GGTCCGCGCCTTCAGCTGCATCTCGGCCTGCGGGCCCC
SEQ ID NO:182	MAX.chr12.52 Converted DNA	GGTTTGGGGTTTAGTCGTTCGTTTTTGTCGTTATCGTATTATGTTTTGTTTTTATTTTCGTTTTAGCGTTTTTTGCGGGG TTCGCGTTTTTAGTTGTATTTCGGTTTGCGGGTTTT
SEQ ID NO:183	MAX.chr12.52 Forward Primer	TCGTTCGTTTTTGTCGTTATCG
SEQ ID NO:184	MAX.chr12.52 Reverse Primer	AACCGAAATACAACTAAAAACGC
SEQ ID NO:185	MAX.chr12.52 Flap oligonucleotide	CCACGGACGCGAACCCCGCAA/3C6/
SEQ ID NO:186	MAX.chr16.50 Target DNA	GGAAGGCTGCAGCGAGAGATTTACATATTCATCCGAGCTTAAGGAAGCCGCGATAATGCAGGTACAGCCCGAAACC CACGCCCCCAGACCTTATCTGCGCGCCCCGCC
SEQ ID NO:187	MAX.chr16.50 Converted DNA	GGAAGGTTGTAGCGAGAGATTTATATATTTATTCGAGTTTAAGGAAGTCGCGATAATGTAGGTATAGTTCGAAATTT ACGTTTTTAGATTTTATTTGCGCGTTTCGTT
SEQ ID NO:188	MAX.chr16.50 Forward Primer	TTCGAGTTTAAGGAAGTCG
SEQ ID NO:189	MAX.chr16.50 Reverse Primer	TCTAAAAACGTAAATTTCGAACT
SEQ ID NO:190	MAX.chr16.50 Flap Oligonucleotide	CCACGGACGGCGATAATGTAG/3C6/
SEQ ID NO:191	MAX.chr19.16 Target DNA	GGAGTTATTTTTAACCATCGCCTCCCAGAACATTACGGAGCTTCCTCTCTCCAACACGCAGGAAACCCTACTTGGCTG TGCTTCCTGCTAACACGAGGCCCTGCGATTGCTGAGAACAACAGCCCCGAGACTGCGCG
SEQ ID NO:192	MAX.chr19.16 Converted DNA	GGAGTTATTTTTAATTATCGTTTTTTAGAATATTACGGAGTTTTTTTTTTTTTAATACGTAGGAAATTTTATTTGGTTGTG TTTTTTGTTAATACGAGGTTTTGCGATTGTTGAGAATAATAGTTTCGAGATTGCGCG

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:193	MAX.chr19.16 Forward Primer	TTTAATTATCGTTTTTTAGAATATTACGGA
SEQ ID NO:194	MAX.chr19.16 Reverse Primer	ACTATTATTCTCAACAATCGCAAAAC
SEQ ID NO:195	MAX.chr19.16 Flap oligonucleotide	CCACGGACGCCTCGTATTAAC/3C6/
SEQ ID NO:196	MAX.chr19.37 Target DNA	GGCGGGCGCTTGGCCAAACAGCCCAAGACTGCGGAATCACACTCGCCACTGTGTACCTGGACGCCATCTGCAGACC CAGCGCCTGCGGGGATTCCGGAAACGGGAGAGCGGGCTTCC
SEQ ID NO:197	MAX.chr19.37 Converted DNA	GGCGGGCGTTTGGTTAAATAGTTTAAGATTGCGGAATTATATTCGTTATTGTGTATTTGGACGTTATTTGTAGATTTA GCGTTTGCGGGGATTTCGGAAACGGGAGAGCGGGTTTTT
SEQ ID NO:198	MAX.chr19.37 Forward Primer	AGTTTAAGATTGCGGAATTATATTCGT
SEQ ID NO:199	MAX.chr19.37 Reverse Primer	TTCCGAAATCCCCGCAA
SEQ ID NO:200	MAX.chr19.37 Flap oligonucleotide	CGCCGAGGAACGCTAAATCT/3C6/
SEQ ID NO:201	MAX_Chr8.124 Target DNA	CGCAGGCTGAGGCCCTCGGGTCCCCAGCGGGTCCTCGCCATCAGTCACTCTCTACGGGCCAGGCCTGGGGGTCACG GCCTGCAGGAGCCTCCCTGCGCGGCCCCACTCCCTCATCTGCGACCCCGTGGGGAGGCGACCCTGACCACCCTCGTT CCG
SEQ ID NO:202	MAX_Chr8.124 Converted DNA	CGTAGGTTGAGGTTTTCGGGTTTTTAGCGGGTTTTCGTTATTAGTTATTTTTTACGGGTTAGGTTTGGGGGTTACGGT TTGTAGGAGTTTTTTTGCGCGGTTTTATTTTTTTATTTGCGATTTCGTGGGGAGGCGATTTTGATTATTTTCGTTTCG
SEQ ID NO:203	MAX_Chr8.124 Forward Primer	GGTTGAGGTTTTCGGGTTTTTAG
SEQ ID NO:204	MAX_Chr8.124 Reverse Primer	CCTCCCCACGAAATCGC
SEQ ID NO:205	MAX_Chr8.124 Flap oligonucleotide	CGCCGAGGGCGGGTTTTCGT/3C6/
SEQ ID NO:206	MAX_Chr8.124 Forward Primer v2	AGGAGTTTTTTTGCGCGG
SEQ ID NO:207	MAX_Chr8.124 Reverse Primer v2	ACGAAAATAATCAAAATCGCCTCC
SEQ ID NO:208	MAX_Chr8.124 Flap oligonucleotide v2	CGCCGAGGCCCACGAAATCG/3C6/
SEQ ID NO:209	MAX_Chr8.145 Target DNA	CGGGGGAGGGCGGCATCAGCCAGAGCCTCAGCCGACGGCGCTCCCCAGGTCCACTTCCCGCTCCGATACCCTCCCC CTAAGCACGATACCCAGGGCCCAGGGCTGCTCTTGGCG
SEQ ID NO:210	MAX_Chr8.145 Converted DNA	CGGGGGAGGGCGGTATTAGTTAGAGTTTTAGTCGACGGCGTTTTTTAGGTTTATTTTTCGTTTCGATATTTTTTTTTTA AGTACGATATTTAGGGTTTAGGGTTGTTTTTGGCG
SEQ ID NO:211	MAX_Chr8.145 Forward Primer	GCGGTATTAGTTAGAGTTTTAGTCG
SEQ ID NO:212	MAX_Chr8.145 Reverse Primer	ACAACCCTAAACCCTAAATATCGT
SEQ ID NO:213	MAX_Chr8.145 Flap oligonucleotide	CCACGGACGGACGGCGTTTTT/3C6/
SEQ ID NO:214	MAX_Chr1.110 Target DNA	CTCCGCTCCCCGCAGGCCTGGCCGCGCGACGGGCACCCAGCGGGTTGTTATCAATTATTCAGGCCCCAAGTTCACGG GCACTGCATCCATTTCCCTCGCGTGCGCCC
SEQ ID NO:215	MAX_Chr1.110 Converted DNA	TTTCGTTTTTCGTAGGTTTGGTCGCGCGACGGGTATTTAGCGGGTTGTTATTAATTATTTAGGTTTTAAGTTTACGGG TATTGTATTTATTTTTTTCGCGTGCGTTT
SEQ ID NO:216	MAX_Chr1.110 Forward Primer	TTTCGTAGGTTTGGTCGCG
SEQ ID NO:217	MAX_Chr1.110 Reverse Primer	AACCTAAATAATTAATAACAACCCGC
SEQ ID NO:218	MAX_Chr1.110 Flap oligonucleotide	CCACGGACGGCGACGGGTATT/3C6/
SEQ ID NO:219	NFIX Target DNA	GTGGGCCGGGCGTGACGCGCGGTCAAAGTGCAATGATTTTTCAGTTCGGTTGGCTAAACAGGGTCAGAGCTGAGA GCGAAGCAGAAGG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:220	NFIX Converted DNA	GTGGGTCGGGCGTGACGCGCGGTTAAAGTGTAATGATTTTTTAGTTCGGTTGGTTAAATAGGGTTAGAGTTGAGAG CGAAGTAGAAGG
SEQ ID NO:221	NFIX Forward Primer	TGGTTCGGGCGTGACGCG
SEQ ID NO:222	NFIX Reverse Primer	TCTAACCCTATTTAACCAACCGA
SEQ ID NO:223	NFIX Flap oligonucleotide	CGCCGAGGGCGGTTAAAGTG/3C6/
SEQ ID NO:224	NKX2-6 Target DNA	GGACCTCCTCGGCCCCGCCCCATCCGCCTTCGGGATGCTGCTGAGCCCCGTCACCTCCACCCCCTTCTCGGTCAAGGA CATCCTGCGACTGGAG
SEQ ID NO:225	NKX2-6 Converted DNA	GGATTTTTTCGGTTTCGTTTTATTCGTTTTCGGGATGTTGTTGAGTTTCGTTATTTTTATTTTTTTTTCGGTTAAGGATA TTTTGCGATTGGAG
SEQ ID NO:226	NKX2-6 Forward Primer	GATTTTTTCGGTTTCGTTTTATTCG
SEQ ID NO:227	NKX2-6 Reverse Primer	CAATCGCAAAATATCCTTAACCGA
SEQ ID NO:228	NKX2-6 Flap oligonucleotide	CCACGGACGGTTTTCGGGATG/3C6/
SEQ ID NO:229	OPLAH Target DNA	CTGTCAGTGCTGACCGAGCGCCGCGCCTTCCGGCCATACGGGCTCCACGGTGCGCGGTTCCCCAGCCCTCGCGGCCC TCCCCGCCCCCG
SEQ ID NO:230	OPLAH Converted DNA	TTGTTAGTGTTGATCGAGCGTCGCGTTTTTCGGTTATACGGGTTTTACGGTGCGCGGTTTTTTAGTTTTCGCGGTTTTT TTCGTTTTCG
SEQ ID NO:231	OPLAH Forward Primer	CGTCGCGTTTTTCGGTTATACG
SEQ ID NO:232	OPLAH Reverse Primer	CGCGAAAACTAAAAAACCGCG
SEQ ID NO:233	OPLAH Flap oligonucleotide	CCACGGACGGCACCGTAAAAC/3C6/
SEQ ID NO:234	PARP15 Target DNA	CGGAGTATGGTGAGGAGCGCGGGGGACGGGTGCGGGAAGGGGACAGCAGGGCTGAGCCTGGGGCCCGCAAGAC CCAGCAGCCCGAGCGGGCGCAGAGACCCCACGCCACGCACA
SEQ ID NO:235	PARP15 Converted DNA	CGGAGTATGGTGAGGAGCGCGGGGGACGGGTGCGGGAAGGGGATAGTAGGGTTGAGTTTGGGGTTCGTAAGAT TTAGTAGTTCGAGCGGGCGTAGAGATTTTACGTTACGTATA
SEQ ID NO:236	PARP15 Forward Primer	GGTTGAGTTTGGGGTTCG
SEQ ID NO:237	PARP15 Reverse Primer	CGTAACGTAAAATCTCTACGCCC
SEQ ID NO:238	PARP15 Flap oligonucleotide	CCACGGACGCGCTCGAACTAC/3C6/
SEQ ID NO:239	PRDM14 Target DNA	GGAGAGCAGCCCGCAGAACCTGGCCGCGTACTACACGCCTTTCCCGTCCTATGGACACTACAGAAACAGCCTGGCC ACCGTGGAGGAAGACTTCC
SEQ ID NO:240	PRDM14 Converted DNA	GGAGAGTAGTTCGTAGAATTTGGTCGCGTATTATACGTTTTTTTCGTTTTATGGATATTATAGAAATAGTTTGGTTAT CGTGGAGGAAGATTTTT
SEQ ID NO:241	PRDM14 Forward Primer	GAGTAGTTCGTAGAATTTGGTCG
SEQ ID NO:242	PRDM14 Reverse Primer	CCACGATAACCAAACTATTTCTATAATATCC
SEQ ID NO:243	PRDM14 Flap oligonucleotide	CCACGGACGGCGTATTATACG/3C6/
SEQ ID NO:244	PRDM14 Forward Primer v3	GGAGAGTAGTTCGTAGAATTTGG
SEQ ID NO:245	PRDM14 Reverse Primer v3	CTATTTCTATAATATCCATAAAACGAAAAAAACGT
SEQ ID NO:246	PRDM14 Flap oligonucleotide v3	CCACGGACGGTCGCGTATTAT/3C6/
SEQ ID NO:247	PRKCB_28 Target DNA	GGGAAGGTGCCCTGCGCGCGCGCGCTCACCAGATGAAGTCGGTGCAGTGGCTGCAGAAGGTGGGCTGCTTGAAGA AGCGGGCGGTGAATTTG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:248	PRKCB_28 Converted DNA	GGGAAGGTGTTTTGCGCGCGCGCGTTTATTAGATGAAGTCGGTGTAGTGGTTGTAGAAGGTGGGTTGTTTGAAGA AGCGGGCGGTGAATTTG
SEQ ID NO:249	PRKCB_28 Forward Primer	GGAAGGTGTTTTGCGCG
SEQ ID NO:250	PRKCB_28 Reverse Primer	CTTCTACAACCACTACACCGA
SEQ ID NO:251	PRKCB_28 Flap oligonucleotide	CCACGGACGGCGCGCGTTTAT/3C6/
SEQ ID NO:252	PTGDR Target DNA	GCCTCGGGGCCCGGGGACTCACAATTACGGGCAGAGAACACATAGTGAAGAGCACGGTCATCAGCGCCAGCAGCA GGAGGTGATCCAGCTCCTCCAGGGGCTGAGGG
SEQ ID NO:253	PTGDR Converted DNA	GTTTCGGGGTTCGGGGATTTATAATTACGGGTAGAGAATATATAGTGAAGAGTACGGTTATTAGCGTTAGTAGTAG GAGGTGATTTAGTTTTTTTAGGGGTTGAGGG
SEQ ID NO:254	PTGDR Forward Primer	GGGTTCGGGGATTTATAATTACGG
SEQ ID NO:255	PTGDR Reverse Primer	CCTCCTACTACTAACGCTAATAACC
SEQ ID NO:256	PTGDR Flap oligonucleotide	CCACGGACGCGTACTCTTCAC/3C6/
SEQ ID NO:257	PTGDR_9 Target DNA	GGCGGCTGCAGCGGCACCCGCGCTCCTGCACCAGGGACTGTGCCGAGCCGCGCGCGGACGGGAGGGAAGCGTCCC CTCAG
SEQ ID NO:258	PTGDR_9 Converted DNA	GGCGGTTGTAGCGGTATTCGCGTTTTTGTATTAGGGATTGTGTCGAGTCGCGCGCGGACGGGAGGGAAGCGTTTTT TTAG
SEQ ID NO:259	PTGDR_9 Forward Primer	GTTGTAGCGGTATTCGCG
SEQ ID NO:260	PTGDR_9 Reverse Primer	CTTCTCTCCCGTCCGCGC
SEQ ID NO:261	PTGDR_9 Flap oligonucleotide	CGCCGAGGCGCGACTCGACA/3C6/
SEQ ID NO:262	RASSF1 (Human) Target DNA	TCCAGAAACACGGGTATCTCCGCGTGGTGCTTTGCGGTCGCCGTCGTTGTGGCCGTCCGGGGTGGGGTGTGAGGA GGGGACGAAGGAGGGAAGGAAGGGCAAGGCGGGGGGGGCTCTGCGAGAGCGCGCCCAGCCCCGCCTTC
SEQ ID NO:263	RASSF1 (Human) Converted DNA	TTTAGAAATACGGGTATTTTCGCGTGGTGTTTTGCGGTCGTCGTCGTTGTGGTCGTTCGGGGTGGGGTGTGAGGAG GGGACGAAGGAGGGAAGGAAGGGTAAGGCGGGGGGGGTTTTGCGAGAGCGCGTTTAGTTTCGTTTTT
SEQ ID NO:264	RASSF1 (Human) Forward Primer	AGAAATACGGGTATTTTCGCG
SEQ ID NO:265	RASSF1 (Human) Reverse Primer	CCACAACGACGACGACC
SEQ ID NO:266	RASSF1 (Human) Flap oligonucleotide	CCACGGACGCGCAAAACACCA/3C6/
SEQ ID NO:267	SHOX2 Target DNA	CGGTCGGGCAGGCGGGACGGAGATTACCTGGCTGTCCAGGGGACCTTATGCAGGGTTTGGCCCGAGCCCAGGGGC AGCGAGGGGCGTCTGCGGATGCGGCTCCCTGTGCGGCACAACACC
SEQ ID NO:268	SHOX2 convereted DNA	CGGTCGGGTAGGCGGGACGGAGATTATTTGGTTGTTTAGGGGATTTTATGTAGGGTTTGGTTCGAGTTTAGGGGTA GCGAGGGGCGTTTGCGGATGCGGTTTTTTGTGCGGTATAATATT
SEQ ID NO:269	SHOX2 Forward Primer	GTTCGAGTTTAGGGGTAGCG
SEQ ID NO:270	SHOX2 Reverse Primer	CCGCACAAAAAACCGCA
SEQ ID NO:271	SHOX2 Flap oligonucleotide	CCACGGACGATCCGCAAACGC/3C6/
SEQ ID NO:272	SHROOM1 Target DNA	CCGGAGCACTCGCCGCTGCGCGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAG
SEQ ID NO:273	SHROOM1 Converted DNA	TCGGAGTATTCGTCGTTGCGCGTTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAG
SEQ ID NO:274	SHROOM1 Forward Primer	GGAGTATTCGTCGTTGCG
SEQ ID NO:275	SHROOM1 Reverse Primer	CGAAAACCGCCTACCGC
SEQ ID NO:276	SHROOM1 Flap oligonucleotide	CGCCGAGGGCGTTTTGAAGT/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:277	SKI Target DNA	CCCGGGCCTACGGTCCTCCCGCCACCTCCACGGGGCGGCTGTTGGGGCCCCACCAGGCAGAGCCGTGTTCTCAGGC GTTGGCTCTCATGGAGGTGG
SEQ ID NO:278	SKI Converted DNA	TTCGGGTTTACGGTTTTTTCGTTATTTTTACGGGGCGGTTGTTGGGGTTTTATTAGGTAGAGTCGTGTTTTTAGGCGT TGGTTTTTATGGAGGTGG
SEQ ID NO:279	SKI Forward Primer	ACGGTTTTTTCGTTATTTTTACGGG
SEQ ID NO:280	SKI Reverse Primer	CAACGCCTAAAAACACGACTC
SEQ ID NO:281	SKI Flap oligonucleotide	CGCCGAGGGGCGGTTGTTGG/3C6/
SEQ ID NO:282	S1PR4 Target DNA	GGGCCTGTCCCGTTCCCTGCTCCCCATACAGGCGAGGCTGCGTGCACACAGCTTCCTGTACCCCAGGAGGGCCTGCC TGGCACGCACCCGGTGGCTGCACCATCCACACGCAAGACTGCAACTTCAGATGCTCCGCACGCTGGAGATG
SEQ ID NO:283	S1PR4 Converted DNA	GGGTTTGTTTCGTTTTTTGTTTTTTATATAGGCGAGGTTGCGTGTATATAGTTTTTTGTATTTTAGGAGGGTTTGTTTG GTACGTATTCGGTGGTTGTATTATTTATACGTAAGATTGTAATTTTAGATGTTTCGTACGTTGGAGATG
SEQ ID NO:284	S1PR4 Forward Primer	TTATATAGGCGAGGTTGCGT
SEQ ID NO:285	S1PR4 Reverse Primer	CTTACGTATAAATAATACAACCACCGAATA
SEQ ID NO:286	S1PR4 Flap oligonucleotide	CCACGGACGACGTACCAAACA/3C6/
SEQ ID NO:287	SLC12A8 Target DNA	CGGAGCTAGGAGGGTGGGGCTCGGAGGGCGCAGGAAGAGCGGCTCTGCGAGGAAAGGGAAAGGAGAGGCCGCT TCTGGGAAGGGACCC
SEQ ID NO:288	SLC12A8 Converted DNA	CGGAGTTAGGAGGGTGGGGTTCGGAGGGCGTAGGAAGAGCGGTTTTGCGAGGAAAGGGAAAGGAGAGGTCGTT TTTGGGAAGGGATTT
SEQ ID NO:289	SLC12A8 Forward Primer	TTAGGAGGGTGGGGTTCG
SEQ ID NO:290	SLC12A8 Reverse Primer	CTTTCCTCGCAAAACCGC
SEQ ID NO:291	SLC12A8 Flap oligonucleotide	CCACGGACGGGAGGGCGTAGG/3C6/
SEQ ID NO:292	SOBP Target DNA	GCCCCGGCGGGCCCCGAGGCGGCCGCGGCCTGCAACGTCATCGTGAACGGCACGCGCGG
SEQ ID NO:293	SOBP Converted DNA	GTTTCGGCGGGTTTCGAGGCGGTCGCGGTTTGTAACGTTATCGTGAACGGTACGCGCGG
SEQ ID NO:294	SOBP Forward Primer	TTTCGGCGGGTTTCGAG
SEQ ID NO:295	SOBP Reverse Primer	CGTACCGTTCACGATAACGT
SEQ ID NO:296	SOBP Flap oligonucleotide	CGCCGAGGGGCGGTCGCGGT/3C6/
SEQ ID NO:297	SOBP Flap oligonucleotide v2	CGCCGAGGTTACAAACCGCG/3C6/
SEQ ID NO:431	SOBP Flap oligonucleotide v3	CGCGCCGAGGTTACAAACCGCGACCG/3C6/
SEQ ID NO:298	SPOCK2 Target DNA	CTAGGCGAGATGGTGGAAGGCGTGTCCGTACGGGGGTGGGCTGGGGTCCCCGTGCAGAAGGGCGCGCGAGGACC CAGGCTGGTTTTCCC
SEQ ID NO:299	SPOCK2 Converted DNA	TTAGGCGAGATGGTGGAAGGCGTGTTCGTACGGGGGTGGGTTGGGGTTTTCGTGTAGAAGGGCGCGCGAGGATT TAGGTTGGTTTTTTT
SEQ ID NO:300	SPOCK2 Forward Primer	CGAGATGGTGGAAGGCG
SEQ ID NO:301	SPOCK2 Reverse Primer	GCGCCCTTCTACACGAA
SEQ ID NO:302	SPOCK2 Flap oligonucleotide	CCACGGACGGTGTTCGTACGG/3C6/
SEQ ID NO:303	ST8SIA1 Target DNA	GCGCTGCTGCGCCGCCAGGCAAGGCGAGGGTCCGGGAGAAGGCTCGGCTCCCTCCTAAACATGTGGCCCGTGGCG TCCCCTTGTCCCCTCCGAGCGATGCTCCTGCGCCCTTCGCCGCCTCCCGCGCTGCTGCGCCGCCAGGCAA

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:304	ST8SIA1 Converted DNA	GGCGAGGGTTCGGGAGAAGGTTCGGTTTTTTTTTAAATATGTGGTTCGTGGCGTTTTTTTGTTTTTTTCGAGCGATGT TTTTGCGTTTTTCGTCGTTTTTCGCGTTGTTGCGTCGTTAGGTAA
SEQ ID NO:305	ST8SIA1 Forward Primer	AAATATGTGGTTCGTGGCGTT
SEQ ID NO:306	ST8SIA1 Reverse Primer	ACGCAACAACGCGAAAAAC
SEQ ID NO:307	ST8SIA1 Flap oligonucleotide	CGCCGAGGCGACGAAAAACG/3C6/
SEQ ID NO:308	ST8SIA1_22 Target DNA	ACGAGAAAGAGATCGTGCAGGGGGTGCTGCAACAGGGCACGGCGTGGAGGAGGAACCAGACCGCGGCCAGAGC GTTCAGGTACTCCTGCCCTCGCGGCTCCTCCCCTCTAGCGTCCTTTCCTCCCCGAGTGCAGAGG
SEQ ID NO:309	ST8SIA1_22 Converted DNA	ACGAGAAAGAGATCGTGTAGGGGGTGTTGTAATAGGGTACGGCGTGGAGGAGGAATTAGATCGCGGTTAGAGCG TTTAGGTATTTTTGTTTTCGCGGTTTTTTTTTTTTAGCGTTTTTTTTTTTTCGAGTGTAGAGG
SEQ ID NO:310	ST8SIA1_22 Forward Primer	GGGGTGTTGTAATAGGGTACG
SEQ ID NO:311	ST8SIA1_22 Reverse Primer	CTAAACGCTCTAACCGCGA
SEQ ID NO:312	ST8SIA1_22 Flap oligonucleotide	CCACGGACGGGCGTGGAGGAG/3C6/
SEQ ID NO:313	SP9 Target DNA	CGCGCCGTTGGTCACCTCGCCGGCCGCCAGCGTCGAATGGAAGCCCGACTTGTACCAGGACTCGTACGGGTGCGCC ATGCCCACGCGCGGGTACAGCCCGTCGGCTGCCGTCGTGTG
SEQ ID NO:314	SP9 Converted DNA	CGCGTCGTTGGTTATTTCGTCGGTCGTTAGCGTCGAATGGAAGTTCGATTTGTATTAGGATTCGTACGGGTGCGTTA TGTTTACGCGCGGGTATAGTTCGTCGGTTGTCGTCGTGTG
SEQ ID NO:315	SP9 Forward Primer	TAGCGTCGAATGGAAGTTCGA
SEQ ID NO:316	SP9 Forward Primer Universal	GGTCGTTAGCGTCGAATG
SEQ ID NO:317	SP9 Reverse Primer	GCGCGTAAACATAACGCACC
SEQ ID NO:318	SP9 Flap oligonucleotide	CCACGGACGCCGTACGAATCC/3C6/
SEQ ID NO:319	SUCLG2 Target DNA	GGTTCCTTCCCGTGGGTTCTTAATCGTCTCGCTGACTTCCAGAATGAAACTGCAGACCCTCGCGGTAAAGATGGCGT GACCAGAA
SEQ ID NO:320	SUCLG2 Converted DNA	GGTTTTTTTTCGTGGGTTTTTAATCGTTTCGTTGATTTTTAGAATGAAATTGTAGATTTTCGCGGTAAAGATGGCGTG ATTAGAA
SEQ ID NO:321	SUCLG2 Forward Primer	TCGTGGGTTTTTAATCGTTTCG
SEQ ID NO:322	SUCLG2 Reverse Primer	TCACGCCATCTTTACCGC
SEQ ID NO:323	SUCLG2 Flap oligonucleotide	CCACGGACGCGAAAATCTACA/3C6/
SEQ ID NO:324	SUCLG2 Forward Primer Universal	GGTTTTTTTTCGTGGGTTTTTAATCG
SEQ ID NO:325	SUCLG2 Reverse Primer Universal	CTAATCACGCCATCTTTACCG
SEQ ID NO:326	SUCLG2 Flap oligonucleotide Universal	CCACGGACGGTTTCGTTGATT/3C6/
SEQ ID NO:327	TBX15 Target DNA	GGAGTGAGTGCCTACAACGCGCAGGCCGGACTGATCCCCCGTTGCTGCAGGTTGGTGCCCCAAGCTGCGGGTGCTC GGGCGCCAACTAAAGCCAGCTCTGTCCAGACGCGGAAAG
SEQ ID NO:328	TBX15 Converted DNA	GGAGTGAGTGTTTATAACGCGTAGGTCGGATTGATTTTTCGTTGTTGTAGGTTGGTGTTTTAAGTTGCGGGTGTTCG GGCGTTAATTAAAGTTAGTTTTGTTTAGACGCGGAAAG
SEQ ID NO:329	TBX15 Reg. 1 Forward Primer	CGTAGGTCGGATTGATTTTTCGT
SEQ ID NO:330	TBX15 Reg. 1 Reverse Primer	TCTAAACAAAACTAACTTTAATTAACGCCC
SEQ ID NO:331	TBX15 Reg. 1 Flap oligonucleotide	CCACGGACGCGAACACCCGCA/3C6/
SEQ ID NO:403	TBX15 Reg. 2 Target DNA	GGAAGGAAATTGCGGGTTCCCGTCTGCCTTGTCTCCAGCTTCTCTGCTGAAGCCCGGTAGCAGTGAATGCGCGCTGA CTTTCAGCGACGACTCCTGGAAGCAACGCCA

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:404	TBX15 Reg. 2 Converted DNA	GGAAGGAAATTGCGGGTTTTCGTTTGTTTTGTTTTTAGTTTTTTTGTTGAAGTTCGGTAGTAGTGAATGCGCGTTGAT TTTTAGCGACGATTTTTGGAAGTAACGTTA
SEQ ID NO:332	TBX15 Reg. 2 Forward Primer	AGGAAATTGCGGGTTTTCG
SEQ ID NO:333	TBX15 Reg. 2 Forward Primer Univ.	GGAAGGAAATTGCGGGTTTTC
SEQ ID NO:334	TBX15 Reg. 2 Reverse Primer	CCAAAAATCGTCGCTAAAAATCAAC
SEQ ID NO:335	TBX15 Reg. 2 Flap oligonucleotide	CCACGGACGCGCGCATTCACT/3C6/
SEQ ID NO:336	TRH Target DNA	GGCGGCCGCGACCCCTCCCCGCTGACCTCACTCGAGCCGCCGCCTGGCGCAGATATAAGCGGCGGCCCATCTGAAG AGGGCTCGGCAGGCGCCCGGGGTC
SEQ ID NO:337	TRH Converted DNA	GGCGGTCGCGATTTTTTTTCGTTGATTTTATTCGAGTCGTCGTTTGGCGTAGATATAAGCGGCGGTTTATTTGAAGAG GGTTCGGTAGGCGTTCGGGGTT
SEQ ID NO:338	TRH Forward Primer	TTTCGTTGATTTTATTCGAGTCG
SEQ ID NO:339	TRH Reverse Primer	TCTTCAAATAAACCGCCGC
SEQ ID NO:340	TRH Flap oligonucleotide	CGCCGAGGGTCGTTTGGCGT/3C6/
SEQ ID NO:432	TRH Forward Primer v2	TTTTCGTTGATTTTATTCGAGTCGTC
SEQ ID NO:433	TRH Reverse Primer v2	GAACCCTCTTCAAATAAACCGC
SEQ ID NO:434	TRH Flap oligonucleotide v2	CGCGCCGAGGCGTTTGGCGTAGATATAAGC/3C6/
SEQ ID NO:341	TSC22D4 Target DNA	CGGGTGGTGAAGCTGCCCCACGGCCTGGGAGAGCCTTATCGCCGCGGTCGCTGGACGTGTGTGGATGTTTATGAGC GAGACCTGGAGCCCCACAGCTTCGGCGGACTCCTGGAGGGAA
SEQ ID NO:342	TSC22D4 Converted DNA	CGGGTGGTGAAGTTGTTTTACGGTTTGGGAGAGTTTTATCGTCGCGGTCGTTGGACGTGTGTGGATGTTTATGAGC GAGATTTGGAGTTTTATAGTTTCGGCGGATTTTTGGAGGGAA
SEQ ID NO:343	TSC22D4 Forward Primer	GTTTGGGAGAGTTTTATCGTCG
SEQ ID NO:344	TSC22D4 Reverse Primer	CCTCCAAAAATCCGCCGA
SEQ ID NO:345	TSC22D4 Flap oligonucleotide	CGCCGAGGGCGGTCGTTGGA/3C6/
SEQ ID NO:346	ZDHHC1 Target DNA	GGGGCGGGGGCCGACAGCCCACGCTGGCGCGGCAGGCGCGTGCGCCCGCCGTTTTCGTGAGCCCGAGCAG
SEQ ID NO:347	ZDHHC1 Converted DNA	GGGGTCGGGGTCGATAGTTTACGTTGGCGCGGTAGGCGCGTGCGTTCGTCGTTTTCGTGAGTTCGAGTAG
SEQ ID NO:348	ZDHHC1 Forward Primer	GTCGGGGTCGATAGTTTACG
SEQ ID NO:349	ZDHHC1 Reverse Primer	ACTCGAACTCACGAAAACG
SEQ ID NO:350	ZDHHC1 Flap oligonucleotide	CGCCGAGGGACGAACGCACG/3C6/
SEQ ID NO:351	ZMIZ1 Target DNA	GGAGCCCCCAGCCCCACGCGGGCACACGCAGGGTGGGTGGTCACGCCCGCAGGGTCCGCGAGCGCGGCGCAGAG CGCGGGCCGTGGGAAGTTTCTC
SEQ ID NO:352	ZMIZ1 Converted DNA	GGAGTTTTTAGTTTTACGCGGGTATACGTAGGGTGGGTGGTTACGTTCGTAGGGTTCGCGAGCGCGGCGTAGAGC GCGGGTCGTGGGAAGTTTTTT
SEQ ID NO:353	ZMIZ1 Forward Primer	GTAGGGTGGGTGGTTACG
SEQ ID NO:354	ZMIZ1 Reverse Primer	AACTTCCCACGACCCGC
SEQ ID NO:355	ZMIZ1 Flap oligonucleotide	CGCCGAGGGTTCGTAGGGTT/3C6/
SEQ ID NO:356	ZNF132 Target DNA	GGCGCCGCCATTGCGGTCCTCATTTTGCTGCTGGTGGGTTGGGCTACAGCAGGCCTCTGGAGCCACACCAGGGCAC GGGAGTGGGTGCAGGGACCGTCACCGCGCCTTCACACGCACCATAGTGCCC
SEQ ID NO:357	ZNF132 Converted DNA	GGCGTCGTTATTGCGGTTTTTATTTTGTTGTTGGTGGGTTGGGTTATAGTAGGTTTTTGGAGTTATATTAGGGTACG GGAGTGGGTGTAGGGATCGTTATCGCGTTTTTATACGTATTATAGTGTTT

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:358	ZNF132 Forward Primer	TGGAGTTATATTAGGGTACGGGA
SEQ ID NO:359	ZNF132 Reverse Primer	ACACTATAATACGTATAAAAACGCGATA
SEQ ID NO:360	ZNF132 Flap oligonucleotide	CCACGGACGAACGATCCCTAC/3C6/
SEQ ID NO:361	ZNF329 Target DNA	GGCGGCGAGGGGCGCGTCCGCGGGTGGGTTTCACCTGGGTGGTGGGCATGTCGGGCCCGCTAGGGCGAGGGTCT GGCCAGGGGCGTAGTTCTCCTGGTGGGTGGGGACGCTCCGTGGCGATTGGGGTCACTCCTCTGAGG
SEQ ID NO:362	ZNF329 Converted DNA	GGCGGCGAGGGGCGCGTTCGCGGGTGGGTTTTATTTGGGTGGTGGGTATGTCGGGTTCGTTAGGGCGAGGGTTTG GTTAGGGGCGTAGTTTTTTTGGTGGGTGGGGACGTTTCGTGGCGATTGGGGTTATTTTTTTGAGG
SEQ ID NO:363	ZNF329 Forward Primer	GGTGGTGGGTATGTCGG
SEQ ID NO:364	ZNF329 Reverse Primer	CCAATCGCCACGAAACG
SEQ ID NO:365	ZNF329 Flap oligonucleotide	CCACGGACGGGTTCGTTAGGG/3C6/
SEQ ID NO:366	ZNF671 Target DNA	CCGTGGGCGCGGACAGCTGCCGGGAGCGGCAGGCGTCTCGATCGGGGACGCAGGCACTTCCGTCCCTGCAGAGCA TCAGACGCGTCTCGGGACACTGGGGACAACATCTCCTCCGCG
SEQ ID NO:367	ZNF671 Converted DNA	TCGTGGGCGCGGATAGTTGTCGGGAGCGGTAGGCGTTTCGATCGGGGACGTAGGTATTTTCGTTTTTGTAGAGTAT TAGACGCGTTTCGGGATATTGGGGATAATATTTTTTTCGCG
SEQ ID NO:368	ZNF671 Forward Primer	GTTGTCGGGAGCGGTAGG
SEQ ID NO:369	ZNF671 Reverse Primer	CCAATATCCCGAAACGCGTCT
SEQ ID NO:370	ZNF671 Flap oligonucleotide	CCACGGACGGCGTTTCGATCG/3C6/
SEQ ID NO:371	ZNF781 Target DNA	AAGCTGCGCCCGGAGACGTGGGAGCGTTCTCTTGTTTTCCGAGTGCGCGGACTCATCGGGTCACAGTTTATGCTTTT ATGACGCGGTGAGTCCAGCCACTGATTCCTAACGGTTTAGAGT
SEQ ID NO:372	ZNF781 Converted DNA	AAGTTGCGTTCGGAGACGTGGGAGCGTTTTTTTGTTTTTCGAGTGCGCGGATTTATCGGGTTATAGTTTATGTTTTTA TGACGCGGTGAGTTTAGTTATTGATTTTTAACGGTTTAGAGT
SEQ ID NO:373	ZNF781 Forward Primer	CGTTTTTTTGTTTTTCGAGTGCG
SEQ ID NO:374	ZNF781 Reverse Primer	TCAATAACTAAACTCACCGCGTC
SEQ ID NO:375	ZNF781 Flap oligonucleotide	CCACGGACGGCGGATTTATCG/3C6/
SEQ ID NO:435	ZNF781 Flap oligonucleotide v2	AGGCCACGGACGGCGGATTTATCGGGTTATAGT/3C6/
SEQ ID NO:376	β-actin Target DNA (ACTB)	CTCTGACCTGAGTCTCCTTTGGAACTCTGCAGGTTCTATTTGCTTTTTCCCAGATGAGCTCTTTTTCTGGTGTTTGTCTC TCTGACTAGGTGTCTAAGACAGTGTTGTGGGTGTAGGTACTAACACTGGCTCGTGTGACAAGGCCATGAGGCTGGT GTAAAGCGGCCTTGGAGTGTGTATTAAGTAGGTGCACAGTAGGTCTGAACAGACTCCCCATCCCAAGA
SEQ ID NO:377	β-actin Converted (BTACT)	TTTTGATTTGAGTTTTTTTTGGAATTTTGTAGGTTTTATTTGTTTTTTTTTAGATGAGTTTTTTTTTTGGTGTTTGTTTTTT TGATTAGGTGTTTAAGATAGTGTTGTGGGTGTAGGTATTAATATTGGTTTGTGTGATAAGGTTATGAGGTTGGTGTA AAGTGGTTTTGGAGTGTGTATTAAGTAGGTGTATAGTAGGTTTGAATAGATTTTTTATTTTAAGA
SEQ ID NO:378	β-actin UT Forward primer	CCATGAGGCTGGTGTAAAG
SEQ ID NO:379	β-actin UT Reverse primer	CTACTGTGCACCTACTTAATACAC
SEQ ID NO:380	β-actin UT Probe (Arm 1)	CGCCGAGGGCGGCCTTGGAG/3C6/
SEQ ID NO:381	β-actin BT Forward primer 65	GTGTTTGTTTTTTTGATTAGGTGTTTAAGA
SEQ ID NO:382	β-actin BT Reverse primer 65	CTTTACACCAACCTCATAACCTTATC
SEQ ID NO:383	β-actin BT probe (Arm 3)	GACGCGGAGATAGTGTTGTGG/3C6/

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FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:384	B3GALT6 Target DNA	GGCCACACAGGCCCACTCTGGCCCTCTGAGCCCCCGGCGGACCCAGGGCATTCAAGGAGCGGCTCTGGGCTGCCAG CGCAGGCCTCCGCGCAAACACAGCAGGCTGGAAGTGGCGCTCATCACCGGCACGTCTTCCCAG
SEQ ID NO:385	B3GALT6 Converted DNA	GGTTATATAGGTTTATTTTGGTTTTTTGAGTTTTCGGCGGATTTAGGGTATTTAAGGAGCGGTTTTGGGTTGTTAGCG TAGGTTTTCGCGTAAATATAGTAGGTTGGAAGTGGCGTTTATTATCGGTACGTTTTTTTAG
SEQ ID NO:386	B3GALT6 Forward Primer	GGTTTATTTTGGTTTTTTGAGTTTTCGG
SEQ ID NO:387	B3GALT6 Reverse Primer	TCCAACCTACTATATTTACGCGAA
SEQ ID NO:388	B3GALT6 Flap oligonucleotide (Arm 5)	CCACGGACGGCGGATTTAGGG/3C6/
SEQ ID NO:436	B3GALT6 Flap oligonucleotide v2 (Arm 5)	ACGGACGCGGAGGCGGATTTAGGGTATTTAAGGAG/3C6/
SEQ ID NO:389	Zebrafish (ZF) Synthetic Target DNA (RASSF1)	TCCAC/iMe-dC/GTGGTGCCCACTCTGGACAGGTGGAGCAGAGGGAAGGTGGTG/iMe-dC/GCATGGTGGG/iMe-dC/GAG/iMe-dC/G/iMe-dC/GTG/iMe-dC/GCCTGGAGGACCC/iMe-dC/GATTGGCTGA/iMe-dC/GTGTAAACCAGGA/iMe-dC/GAGGACATGACTTTCAGCCCTGCAGCCAGACACAGCTGAGCTGGTGTGACCTGTGTGGAGAGTTCATCTGG
SEQ ID NO:390	Zebrafish (ZF) Convert. Synthetic Target DNA	TTTATCGTGGTGTTTATTTTGGATAGGTGGAGTAGAGGGAAGGTGGTGCGTATGGTGGGCGAGCGCGTGCGTTTG GAGGATTTCGATTGGTTGACGTGTAAATTAGGACGAGGATATGATTTTTAGTTTTGTAGTTAGATATAGTTGAGTTG GTGTGATTTGTGTGGAGAGTTTATTTGG
SEQ ID NO:391	ZF_RASSF1 UT Forward primer	CGCATGGTGGGCGAG
SEQ ID NO:392	ZF_RASSF1 UT Reverse primer	ACACGTCAGCCAATCGGG
SEQ ID NO:393	ZF_RASSF1 UT Probe (Arm 3)	GACGCGGAGGCGCGTGCGCC/3C6/
SEQ ID NO:394	ZF_RASSF1 BT Forward primer	TGCGTATGGTGGGCGAG
SEQ ID NO:395	ZF_RASSF1 BT Reverse primer	CCTAATTTACACGTCAACCAATCGAA
SEQ ID NO:396	ZF_RASSF1 BT probe (Arm 3)	GACGCGGAGGCGCGTGCGTTT/3C6/
SEQ ID NO:397	ZF_RASSF1 BT probe (Arm 5)	CCACGGACGGCGCGTGCGTTT/3C6/
SEQ ID NO:398	Arm 1 QUASAR-670 FRET cassette	Q670-TCT-BHQ-2-AGCCGGTTTTCCGGCTGAGACCTCGGCG/3C6/
SEQ ID NO:399	Arm 1 HEX FRET cassette	/HEX/ TCT/BHQ-1/AGCCGGTTTTCCGGCTGAGACCTCGGCG/3C6/
SEQ ID NO:400	Arm 3 QUASAR-670 FRET cassette	Q670-TCT-BHQ-2/AGCCGGTTTTCCGGCTGAGACTCCGCGTC/3C6
SEQ ID NO:401	Arm 5 FAM FRET cassette	d-FAM-TCT-BHQ-1-AGCCGGTTTTCCGGCTGAGACGTCCGTGG/3C6/
SEQ ID NO:402	Arm 7 FAM FRET cassette	d-FAM-TCT-BHQ-1-AGCCGGTTTTCCGGCTGAGAGGACGCGC/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:437	FPR1 mRNA transcript variant 1	AAUCAUUAGAGCCUGAGUCACUCUCCCCAGGAGACCCAGACCUAGAACUACCCAGAGCAAGACCACAGCUGGUG AACAGUCCAGCCUGUCUCCAGUUGGACUAGCCACAAUUCAAGUGCUUGAAAACCACAUGUGGAGCAGACAAGA UGGAGACAAAUUCCUCUCUCCCCACGAACAUCUCUGGAGGGACACCUGCUGUAUCUGCUGGCUAUCUCUUCCU GGAUAUCAUCACUUAUCUGGUAUUUGCAGUCACCUUUGUCCUCGGGGUCCUGGGCAACGGGCUUGUGAUCU GGGUGGCUGGAUUCCGGAUGACACACACAGUCACCACCAUCAGUUACCUGAACCUGGCCGUGGCUGACUUCUG UUUCACCUCCACUUUGCCAUUCUUCAUGGUCAGGAAGGCCAUGGGAGGACAUUGGCCUUUCGGCUGGUUCCU GUGCAAAUUCGUCUUUACCAUAGUGGACAUCAACUUGUUCGGAAGUGUCUUCCUGAUCGCCCUCAUUGCUCU GGACCGCUGUGUUUGCGUCCUGCAUCCAGUCUGGACCCAGAACCACCGCACCGUGAGCCUGGCCAAGAAGGUG AUCAUUGGGCCCUGGGUGAUGGCUCUGCUCCUCACAUUGCCAGUUAUCAUUCGUGUGACUACAGUACCUGGU AAAACGGGGACAGUAGCCUGCACUUUUAACUUUUCGCCCUGGACCAACGACCCUAAAGAGAGGAUAAAUGUG GCCGUUGCCAUGUUGACGGUGAGAGGCAUCAUCCGGUUCAUCAUUGGCUUCAGCGCACCCAUGUCCAUCGUU GCUGUCAGUUAUGGGCUUAUUGCCACCAAGAUCCACAAGCAAGGCUUGAUUAAGUCCAGUCGUCCCUUACGG GUCCUCUCCUUUGUCGCAGCAGCCUUUUUUCUCUGCUGGUCCCCAUAUCAGGUGGUGGCCCUUAUAGCCACA GUCAGAAUCCGUGAGUUAUUGCAAGGCAUGUACAAAGAAAUUGGUAUUGCAGUGGAUGUGACAAGUGCCCU GGCCUUCUUCAACAGCUGCCUCAACCCCAUGCUCUAUGUCUUCAUGGGCCAGGACUUCCGGGAGAGGCUGAUC CACGCCCUUCCCGCCAGUCUGGAGAGGGCCCUGACCGAGGACUCAACCCAAACCAGUGACACAGCUACCAAUUC UACUUUACCUUCUGCAGAGGUGGAGUUACAGGCAAAGUGAGGAGGGAGCUGGGGGACACUUUCGAGCUCCC AGCUCCAGCUUCGUCUCACCUUGAGUUAGGCUGAGCCACAGGCAUUUCCUGCUUAUUUUAGGAUUACCCACU CAUCAGAAAAAAAAAAAAAAGCCUUUGUGUCCCCUGAUUUGGGGAGAAUAAACAGAUAUGAGUUUAUUA
SEQ ID NO:438	FPR1 synthetic standard RNA	GUUGCCAUGUUGACGGUGAGAGGCAUCAUCCGGUUCAUCAUUGGCUUCAGCGCACCCAUGUCCAUCGUUGCU GUCAGUUAUGGGCUUAUUGCCACCAAGAUCCACA
SEQ ID NO:439	FPR1 Forward primer	TGACGGTGAGAGGCATCA
SEQ ID NO:440	FPR1 Reverse primer	GGTGGCAATAAGCCCATAACTG
SEQ ID NO:441	FPR1 hydrolysis probe	CGGTTCATCATTGGCTTCAGCGC
SEQ ID NO:442	NY-ESO-1 antigen protein	MQAEGRGTGGSTGDADGPGGPGIPDGPGGNAGGPGEAGATGGRGPRGAGAARASGPGGGAPRGPHGGAASGLNG CCRCGARGPESRLLEFYLAMPFATPMEAELARRSLAQDAPPLPVPGVLLKEFTVSGNILTIRLTAADHRQLQLSISSCLQQLS LLMWITQCFLPVFLAQPPSGQRR

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FIG. 7

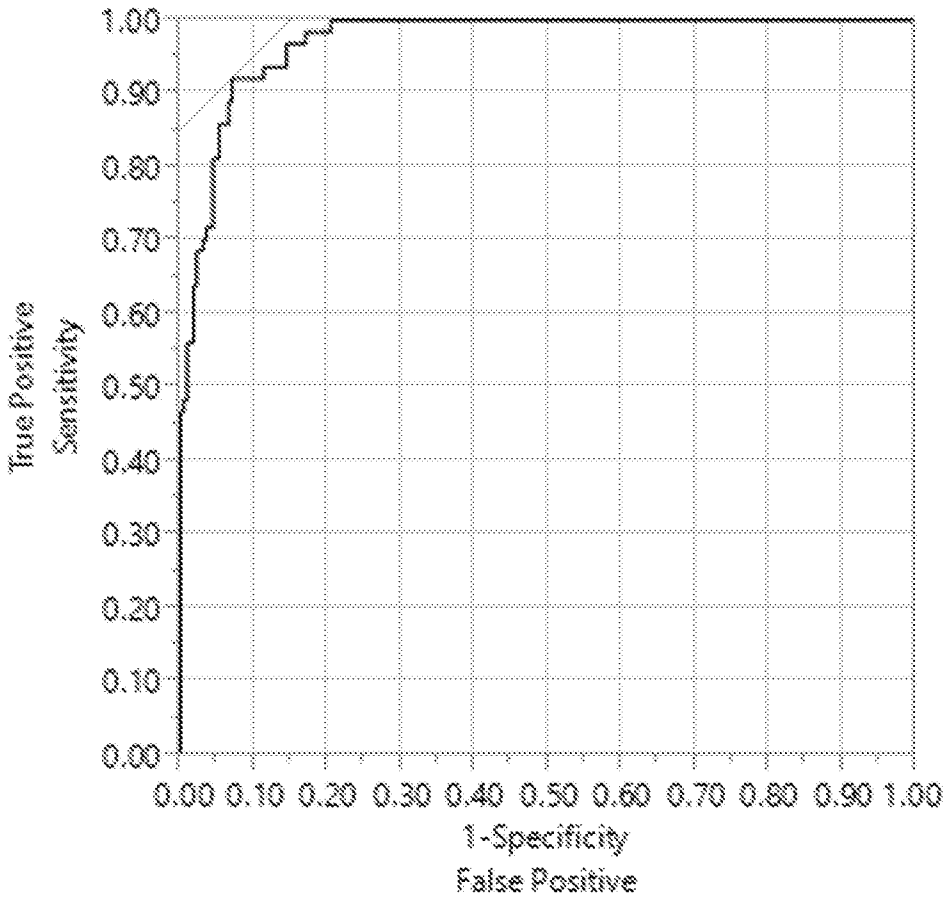

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
True Positive
Sensitivity
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
1-Specificity
False Positive

FIG. 8

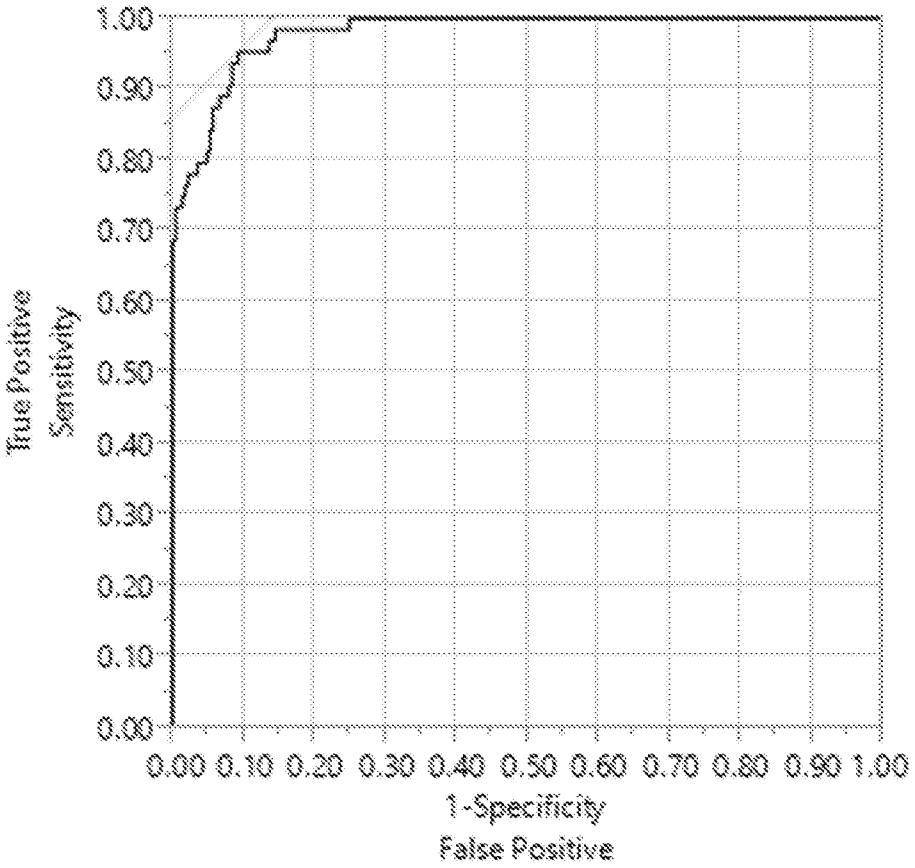

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
True Positive
Sensitivity
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
1-Specificity
False Positive

FIG. 9A

BARX1

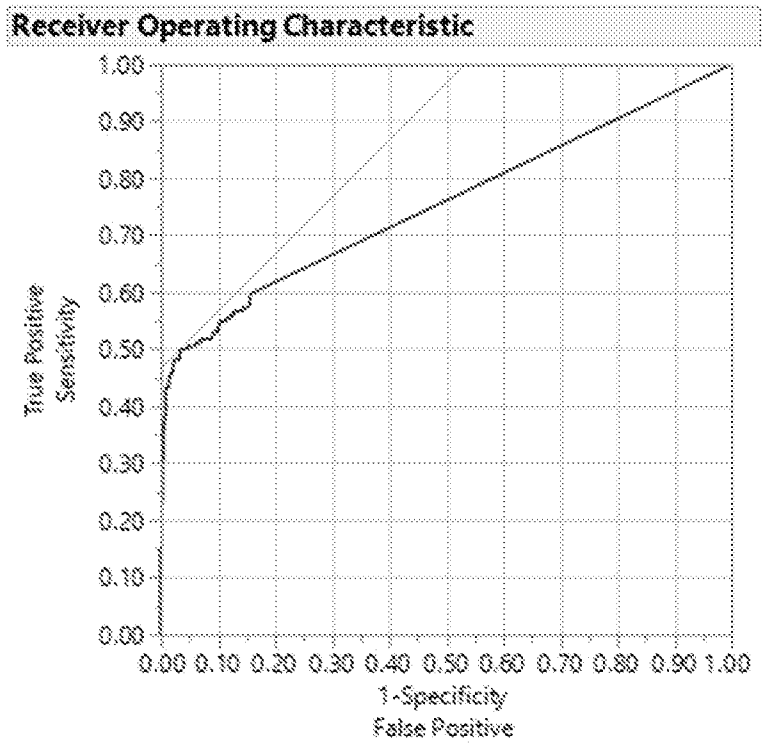
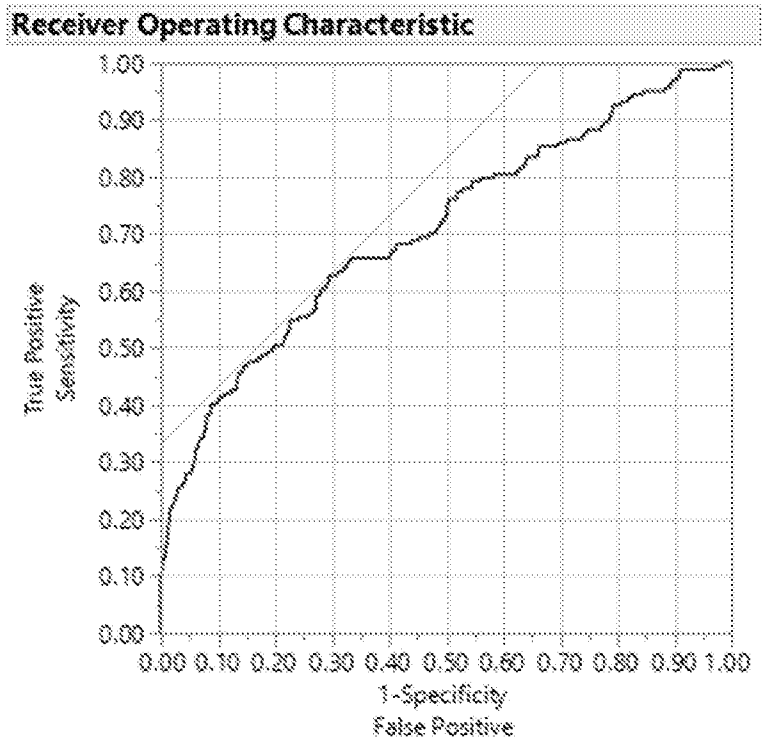

Receiver Operating Characteristic
True Positive
Sensitivity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
1-Specificity
False Positive
Using Cat='A' to be the positive level
AUC
0.75366

FIG. 9B

FLJ45983

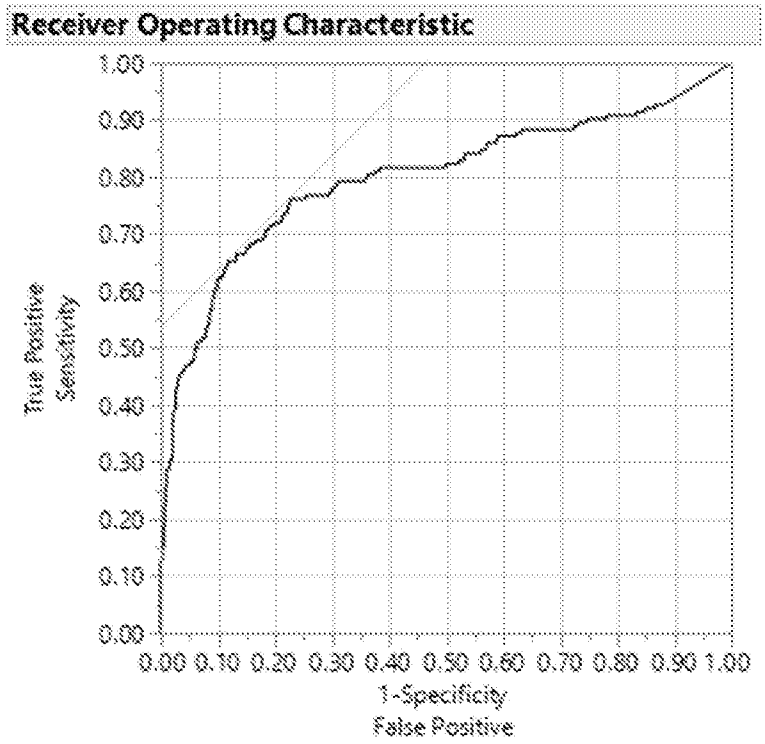


Using Cat='A' to be the positive level

AUC
0.70850

FIG. 9C

HOXA9

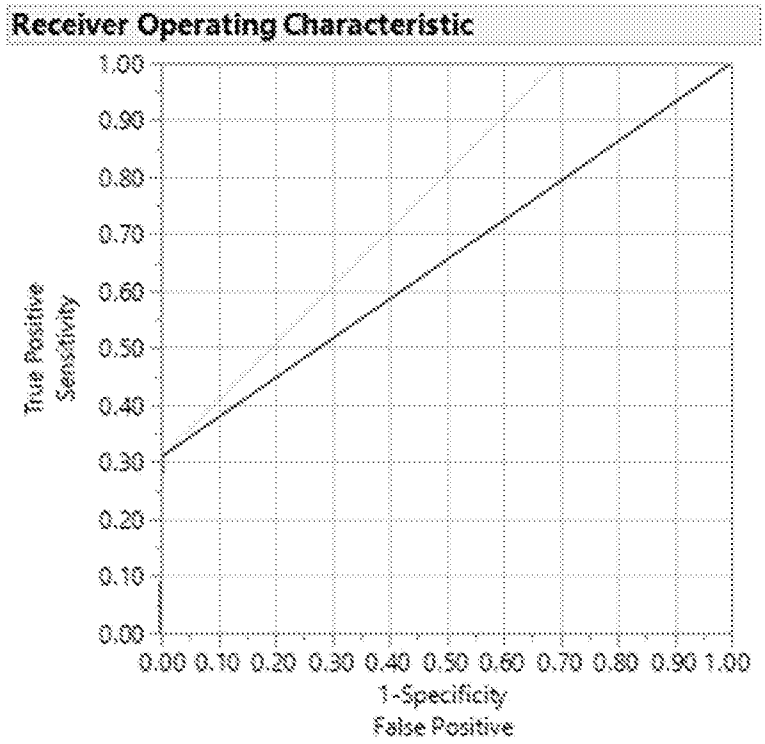


Using Cat='A' to be the positive level

AUC
0.80013

FIG. 9D

HOPX

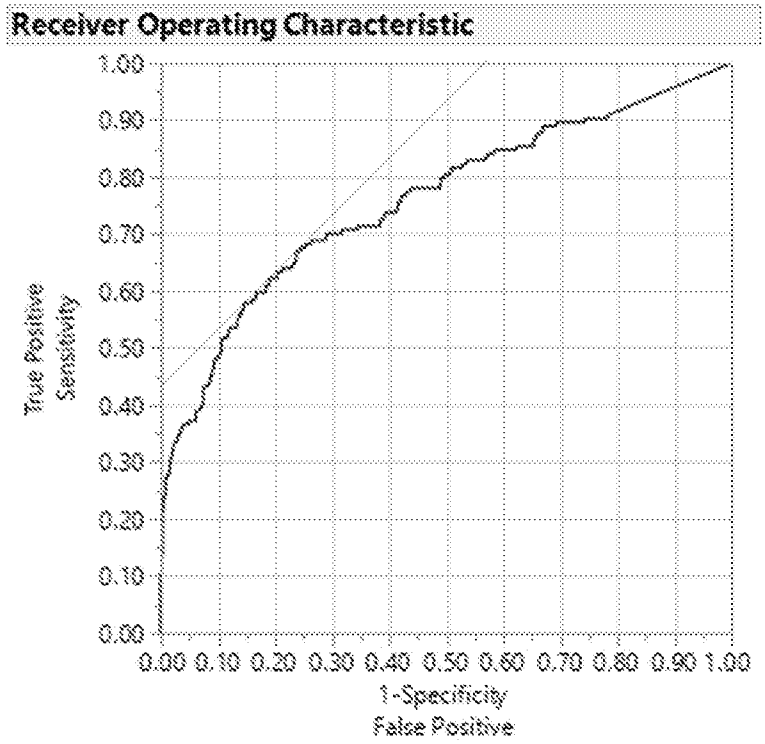


Using Cat='A' to be the positive level

AUC
0.65420

FIG. 9E

ZNF781

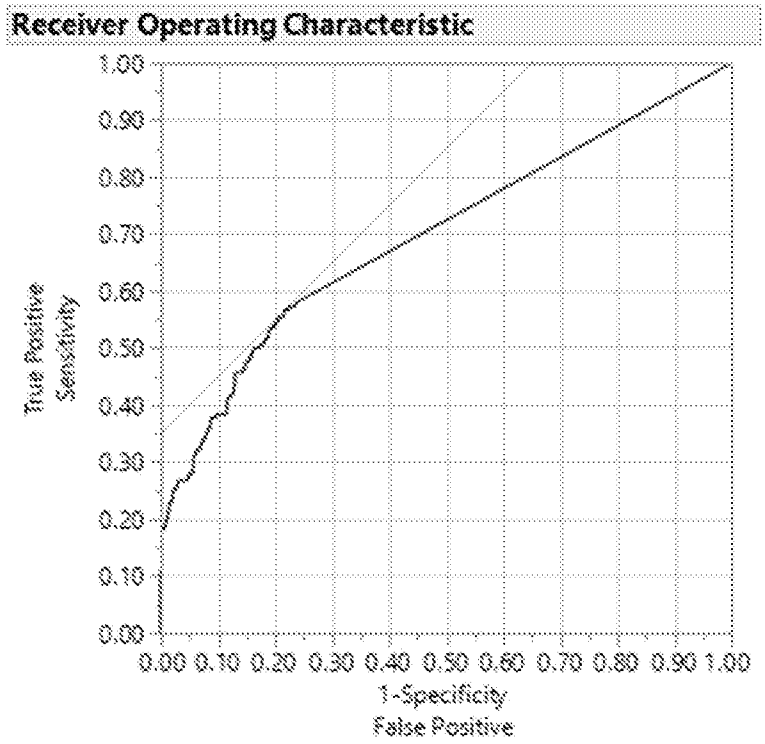


Using Cat='A' to be the positive level

AUC
0.75998

FIG. 9F

HOXB2

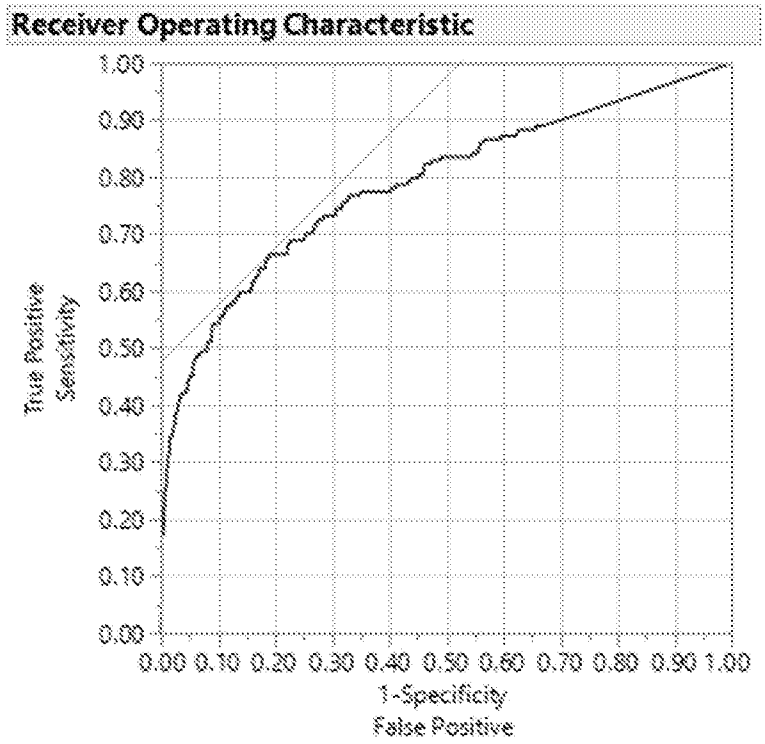


Using Cat='A' to be the positive level

AUC
0.69889

FIG. 9G

IFFO1



Using Cat='A' to be the positive level

AUC
0.78814

FIG. 9H

SOBP

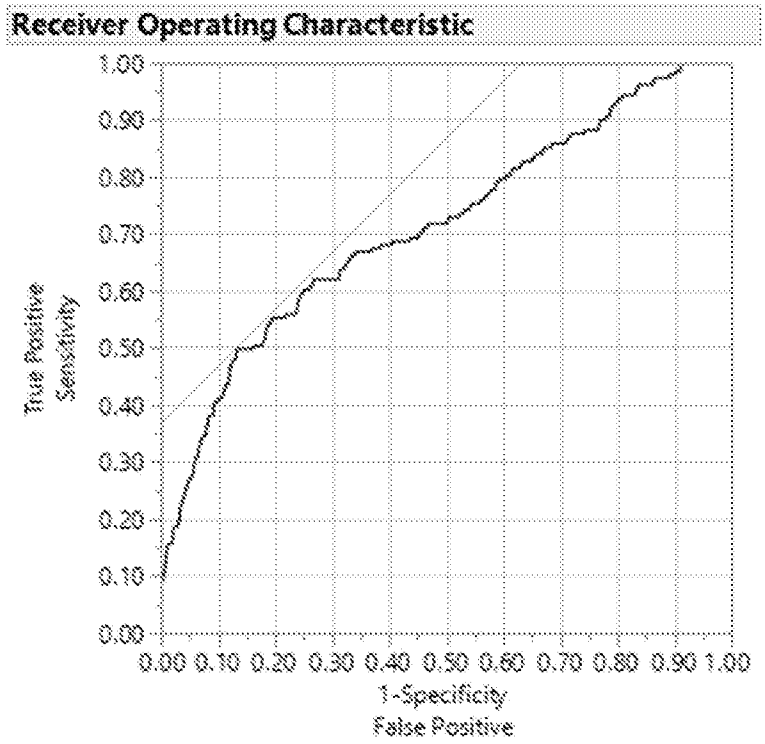

Receiver Operating Characteristic
True Positive Sensitivity
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
0.00 0.10 0.20 0.30 0.40 0.50 0.60 0.70 0.80 0.90 1.00
1-Specificity
False Positive
Using Cat='A' to be the positive level
AUC
0.71690

FIG. 9I

FAM59B

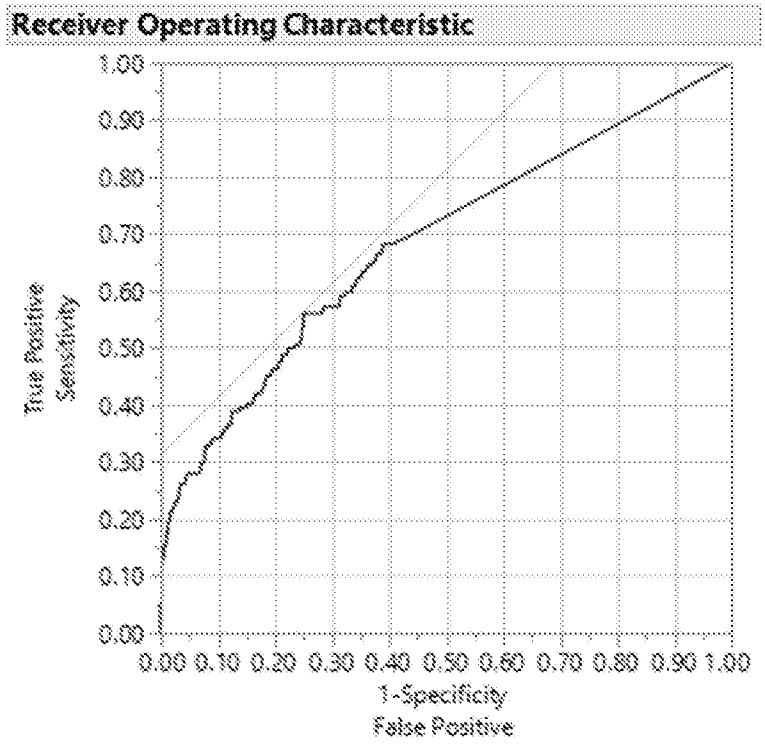


FIG. 10

BARX1, FLJ45983, SOBP, HOPX, IFFO1, and ZNF781