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(54) Title: DETECTION OF LUNG NEOPLASIA BY ANALYSIS OF METHYLATED DNA

(57) Abstract: Provided herein is technology for lung neoplasia screening and particularly, but not exclusively, to methods, compositions, and related uses for detecting the presence of lung cancer.



DETECTION OF LUNG NEOPLASIA BY ANALYSIS OF METHYLATED DNA

The present application claims priority to U.S. Provisional Application Serial Nos. 62/332,295, filed May 5, 2016 and 62/462,677, filed February 23, 2017, each of which is incorporated herein by reference.

5 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

10 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.

15 SUMMARY OF THE INVENTION

Provided herein is a collection of methylated methylation markers assayed on tissue 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
 20 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
 25 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*,
 30 *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, SIPR4, SKI,
 5 *SUCLG2, TBX15, ZDHHC1, and ZNF329* 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
 10 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
 15 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 6 markers (*SHOX2, SOBP, ZNF781, CYP26C1, SUCLG2, and SKI* gave an ROC) resulted in a sensitivity of 92.2% at 93% specificity, and a panel of 4 markers (*ZNF781, BARX1, EMX1,*
 20 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 methylated marker and of a
 25 reference marker in the sample, and comparing the amount of the methylated 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
 30 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, SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329.

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*.

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*,
5 *SPOCK2*, *TSC22D4*, *MAX.chr8.124*, *RASSF1*, *ZNF671*, *ST8SLA1*, *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*,
10 *CYP26C1*, *ZNF781*, *PTGDR*, *GRIN2D*, *MATK*, *BCAT1*, *PRKCB_28*, *ST8SLA_22*, *FLJ45983*,
DLX4, *SHOX2*, *EMX1*, *HOXB2*, *MAX.chr12.526*, *BCL2L11*, *OPLAH*, *PARP15*, *KLHDC7B*,
SLC12A8, *BHLHE23*, *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*,
NFIX, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329* in a sample obtained from a subject;
- b) assaying said sample for an amount of reference marker in said sample;
- 15 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
20 the presence or absence of a lung neoplasm in said subject.

In some embodiments, the sample is assayed for at least two of the markers, and preferably the at least two methylated marker genes are selected from the group consisting of *SLC12A8*, *KLHDC7B*, *PARP15*, *OPLAH*, *BCL2L11*, *MAX.chr12.526*, *HOXB2*, *EMX1*
25 *CYP26C1*, *SOBP*, *SUCLG2*, *SHOX2*, *ZDHHC1*, *NFIX*, *FLJ45983*, *HOXA9*, *B3GALT6*,
ZNF781, *SP9*, *BARX1*, and *SKI*. 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*,
30 *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 the method used for assaying comprises obtaining a sample comprising DNA from a subject, and treating DNA obtained from the sample with a reagent that selectively modifies unmethylated cytosine residues in the obtained DNA to produce modified residues. In preferred embodiments the reagent comprises a bisulfite reagent.

In some embodiments assaying 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.

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 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-sensitive 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 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*, *ST8SLA1*, *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*, *ST8SLA_22*, *FLJ45983*, *DLX4*, *SHOX2*, *EMX1*, *HOXB2*, *MAX.chr12.526*, *BCL2L11*, *OPLAH*, *PARP15*, *KLHDC7B*, *SLC12A8*, *BHLHE23*, *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*, *NFIX*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*. 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 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 at least one oligonucleotide in the kit is selected to
 5 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,
 10 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
 15 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.

20 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*,
 25 *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*,
 30 *NFIX*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329* 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
 5 (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,
 10 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.

In some embodiments, the mixture comprises bisulfite-converted target nucleic acid
 15 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, and 404.

20 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.

25 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.

30 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.

5 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.”

10 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
15 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
20 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
25 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
30 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 or 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 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.*

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

5 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.

15 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.

25 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.

30 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 $R = (A \times B) / (C \times D)$, 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 terms “methylation-specific restriction enzyme” or “methylation-sensitive restriction enzyme” refers to an 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, the cut will not take place or will take place with a significantly reduced efficiency if the recognition site is methylated. In the case of a restriction enzyme that specifically cuts if the recognition site is methylated, 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
5 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,
10 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
15 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,
20 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
25 presence of the target nucleic acid can be detected. A “segment” is defined as a region of nucleic acid within the target sequence.

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
30 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 entirety), 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 entirety), 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 entirety), 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; and 9,212,392, 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
5 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,
10 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
15 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
20 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,
25 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
30 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).

5 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
10 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.

15 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
20 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
25 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
30 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.*,
5 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
10 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
15 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.
20 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
25 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,
30 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.

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.

5 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.

10 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
15 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
20 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.

25 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
30 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

5 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*

10 *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*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, preferably to a marker selected from the subset

15 *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*,

20 *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*,

25 *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*,

30 *DLX4*, *SHOX2*, *EMX1*, *HOXB2*, *MAX.chr12.526*, *BCL2L11*, *OPLAH*, *PARP15*, *KLHDC7B*, *SLC12A8*, *BHLHE23*, *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*, *NFIX*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, 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*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, 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*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, 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, SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329, preferably from any

5 of the subsets of markers as recited above, and a methylation-sensitive 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,

10 *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,

15 *SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329, 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

20 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,

25 *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,

SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329, preferably from any of the subsets of markers as recited above, ; comparing the methylation state of the marker from the subject

30 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*,
5 *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*,
10 *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*, *NFIX*, *S1PR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, 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
15 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
20 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
25 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*,
30 *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 ZNF329, 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, and ZNF329*, 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, and ZNF329*, 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, SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329, 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, SIPR4, SKI, SUCLG2, TBX15, ZDHHC1, and ZNF329, 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, and ZNF329, preferably from any of the subsets of markers as recited above; and c)

- 5 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
 10 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
 15 methylation-sensitive arbitrarily primed PCR (Gonzalzo 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-sensitive restriction enzymes followed by Southern analysis of the regions of interest (digestion-Southern method). In some embodiments, analyzing changes in methylation patterns involves a PCR-based process
 20 that involves digestion of genomic DNA with methylation-sensitive restriction enzymes prior to PCR amplification (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
 25 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**:
 30 160–163). 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).

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 amount 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 methylated 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 (Gonzalzo & 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; Zeschngk 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-sensitive restriction 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
5 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
10 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.*,
15 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,
20 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 (Gonzalzo & Jones, Nucleic Acids Res. 25:2529-2531, 1997), methylation-specific PCR (“MSP”; Herman et al., Proc. Natl. Acad. Sci. USA
25 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
30 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-sensitive 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, overlies 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
5 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”
10 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
15 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
20 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).
25 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)
30 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
5 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
10 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
15 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
20 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
25 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
30 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
5 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
10 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
15 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
20 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
25 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
30 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
5 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
10 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
15 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
20 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
25 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
30 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 (5 (*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*, *ST8SLA1*, *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*, 10 *DMRTA2*, *ARHGEF4*, *CYP26C1*, *ZNF781*, *PTGDR*, *GRIN2D*, *MATK*, *BCAT1*, *PRKCB_28*, *ST8SLA_22*, *FLJ45983*, *DLX4*, *SHOX2*, *EMX1*, *HOXB2*, *MAX.chr12.526*, *BCL2L11*, *OPLAH*, *PARP15*, *KLHDC7B*, *SLC12A8*, , *BHLHE23*, *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*, *NFIX*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*) are used. In some embodiments, an assay further comprises detection of a 15 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 20 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 25 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 30 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
5 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
10 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
15 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
20 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
25 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
30 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.

5 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
10 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
15 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
20 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
25 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
30 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 methylated 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 μ L of Proteinase K (20mg/mL) is added and mixed.
- Add 3 μ L of 1 μ g/ μ L 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 3 mL of plasma lysis buffer and mix.
- Add 200 μ L magnetic silica binding beads (16 μ g of beads/ μ L} and mix again.
- Add 2 mL of 100% isopropanol and mix.
- 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 750 μ L 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 μ L 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 μ L 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.
- 5 • Add 250 μ L 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 μ L 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.
- 10 • Dry the beads at 70°C for 15 minutes, with shaking.
- Add 125 μ L 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.
- 15 • 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 μ L DNA, 7 μ L 1 N NaOH, and 9 μ L 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 μ L 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 μ g/ μ L).
- Binding buffer: 6.5-7 M guanidine hydrochloride.
- 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:

- 5 1. Mix bead stock thoroughly by vortexing bottle for 1 minute.
2. Aliquot 50 μ L of beads into a 2.0 mL tube (*e.g.*, from USA Scientific).
3. Add 750 μ L of binding buffer to the beads.
4. Add 150 μ L of sulfonated DNA from step I.
5. Mix (*e.g.*, 1000 RPM at 30°C for 30 minutes).
- 10 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 μ L 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.
- 15 9. Add 250 μ L 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 μ L 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 μ L of wash buffer. Mix (*e.g.*, 1000 RPM at 30°C for 3 minutes).
- 20 14. Place tube on magnetic rack; remove and discard supernatant after 1 minute.
15. Add 250 μ L 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.
- 25 18. Remove tube from magnetic rack and add 70 μ L 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.

30

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, 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 MgCl₂, 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 MgCl₂, 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
	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
 5 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)
 10 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
 15 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%.
- 20 • 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
 25 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=+
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_Ch1.110	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
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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:
 5 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:
 10 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,
 15 MATK, SHOX2, BCAT1, SUCLG2, BIN2, PRKAR1B, SHROOM1, S1PR4, NFIX, and BTACT.

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

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
 25 PCR was complete, the PCR product was diluted 1:10 using a diluent of 20ng/ μ L of fish DNA (*e.g.*, in water or buffer, see US Pat. No. 9,212,392, incorporated herein by reference) and 10 μ L 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, BCL2L1, 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.

5

The sensitivities for the 51 markers are shown below.

TABLE 2

Marker	Maximum % methylation for normal	Cancer (N=136)		
		# 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).

5 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. BCL2L1
- 10 3. BIN2_Z
4. CYP26C1
5. DLX4
6. DMRTA2
7. DNMT3A
- 15 8. EMX1
9. FERMT3
10. FLJ45983
11. HOXA9
12. KLHDC7B
- 20 13. MAX.chr10.22624430-22624544
14. MAX.chr12.52652268-52652362
15. MAX.chr8.124173236-124173370
16. MAX.chr8.145105646-145105653
17. NFIX
- 25 18. OPLAH
19. PARP15
20. PRKCB_28
21. S1PR4
22. SHOX2
- 30 23. SKI
24. SLC12A8
25. SOBP
26. SP9
27. SUCLG2
- 35 28. TBX15
29. ZDHHC1
30. ZNF781

40 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')
BARX1	BARX1_FP	Forward Primer	CGTTAATTTGTTAGATAGAGGGCG
	BARX1_RP	Reverse Primer	ACGATCGTCCGAACAACC
	BARX1_Pb_A5	Flap Oligo.	CCACGGACGCGCCTACGAAAA/3C6/
SLC12A8	SLC12A8_FP	Forward Primer	GGTTTTTATTTTTGGATTAGCGATCGA
	SLC12A8_RP	Reverse Primer	CGCTAACTATTCTCGATTACGC
	SLC12A8_Pb_A1	Flap Oligo.	CGCCGAGGACGATTAGTTAG/3C6/
PARP15	PARP15_FP	Forward Primer	GGTTGAGTTTGGGGTTCG
	PARP15_RP	Reverse Primer	CGTAACGTAAAACTCTACGCCC
	PARP15_Pb_A5	Flap Oligo.	CCACGGACGCGCTCGAACTAC/3C6/
MAX.Chr8.124	MAX.Chr8.124_FP	Forward Primer	GGTTGAGGTTTTCGGGTTTTAG
	MAX.Chr8.124_RP	Reverse Primer	CCTCCCCACGAAATCGC
	MAX.Chr8.124_Pb_A1	Flap Oligo.	CGCCGAGGGCGGGTTTTCTGT/3C6/
SHOX2	SHOX2_FP	Forward Primer	GTTTCGAGTTTAGGGGTAGCG
	SHOX2_RP	Reverse Primer	CCGCACAAAAAACGCA
	SHOX2_Pb_A5	Flap Oligo.	CCACGGACGATCCGCAACGC/3C6/
ZDHC1	ZDHC1FP	Forward Primer	GTCGGGGTCGATAGTTTACG
	ZDHC1RP_V3	Reverse Primer	ACTCGAACTACGAAAACG
	ZDHC1Probe_v3_A1	Flap Oligo.	CGCCGAGGGACGAACGCACG/3C6/
BIN2_Z	BIN2_FP_Z	Forward Primer	GGGTTTATTTTTAGGTAGCGTTCG
	BIN2_RP_Z	Reverse Primer	CGAAATTTCGAACAAAAATTAATACTCGA
	BIN2_Pb_A5_Z	Flap Oligo.	CCACGGACGGTTCGAGGTTAG/3C6/
SKI	SKI_FP	Forward Primer	ACGGTTTTTCGTTATTTTTACGGG
	SKI_RP	Reverse Primer	CAACGCCTAAAAACACGACTC
	SKI_Pb_A1	Flap Oligo.	CGCCGAGGGGCGGTTGTTGG/3C6/
DNMT3A	DNMT3A_FP	Forward Primer	GTTACGAATAAAGCGTTGGCG
	DNMT3A_RP	Reverse Primer	AACGAAACGTCTTATCGCGA
	DNMT3A_Pb_A5	Flap Oligo.	CCACGGACGGAGTGCGCGTTC/3C6/
BC2L11	BCL2L11_FP	Forward Primer	CGTAATGTTTCGCGTTTTTCG
	BCL2L11_RP	Reverse Primer	ACTTCTTCTACGTAATTCTTTCCGA
	BCL2L11_Pb_A1	Flap Oligo.	CGCCGAGGGCGGGGTCGGGC/3C6/
TBX15	TBX15_Reg2_FP	Forward Primer	AGGAAATTGCGGGTTTTTCG
	TBX15_Reg2_RP	Reverse Primer	CCAAAAATCGTCGCTAAAAATCAAC
	TBX15_Reg2_Pb_A5	Flap Oligo.	CCACGGACGCGCGCATTCACT/3C6/
FERMT3	FERMT3_FP	Forward Primer	GTTTTCGGGGATTATATCGATTCTG
	FERMT3_RP	Reverse Primer	CCCAATAACCCGCAAAATAACC
	FERMT3_Pb_A1	Flap Oligo.	CGCCGAGGCGACTCGACCTC/3C6/
PRKCB_28	PRKCB_28_FP	Forward Primer	GGAAGGTGTTTTGCGCG
	PRKCB_28_RP	Reverse Primer	CTTCTACAACCACTACACCGA
	PRKCB_28_Pb_A5	Flap Oligo.	CCACGGACGGCGCGCGTTTAT/3C6/
SOBP_HM	SOBP_HM_FP	Forward Primer	TTTCGGCGGGTTTCGAG
	SOBP_HM_RP	Reverse Primer	CGTACCGTTACGATAACGT
	SOBP_HM_Pb_A1	Flap Oligo.	CGCCGAGGGGCGGTCGCGGT/3C6/

MAX.chr8.145	MAX.Chr8.145_FP	Forward Primer	GCGGTATTAGTTAGAGTTTTAGTCG
	MAX.Chr8.145_RP	Reverse Primer	ACAACCCTAAACCCTAAATATCGT
	MAX.Chr8.145_Pb_A5	Flap Oligo.	CCACGGACGGACGGCGTTTTT/3C6/
MAX.chr10.226	MAX.Chr10.226_FP	Forward Primer	GGGAAATTTGTATTTCTGTAATATCG
	MAX.Chr10.226_RP	Reverse Primer	ACAATAACTTATCTACGTAACATCGT
	MAX_Chr10.226_Pb_A1	Flap Oligo.	CGCCGAGGGCGGTTAAGAAA/3C6/
MAX.chr12.52	MAX.Chr12.52_FP	Forward Primer	TCGTTCTGTTTTGTCTGTATCG
	MAX.Chr12.52_RP	Reverse Primer	AACCGAAATACAATAAAAAACGC
	MAX.Chr12.52PbA1	Flap Oligo.	CCACGGACGCGAACCCCGCAA/3C6/
FLJ45983	FLJ45983_FP	Forward Primer	GGGCGCGAGTATAGTCG
	FLJ45983_RP	Reverse Primer	CAACGCGACTAATCCGC
	FLJ45983_Pb_A1	Flap Oligo.	CGCCGAGGCCGTCACTCCA/3C6/
HOXA9	HOXA9_FP	Forward Primer	TTGGGTAATTATTACGTGGATTCTG
	HOXA9_RP	Reverse Primer	ACTCATCCGCGACGTC
	HOXA9_Pb_A5	Flap Oligo.	CCACGGACGCGACGCCCAACA/3C6/
EMX1	EMX1_FP	Forward Primer	GGCGTCGCGTTTTTTAGAGAA
	EMX1_RP	Reverse Primer	TTCCTTTTCGTTCTGTATAAAATTTCTGT
	EMX1PbA1	Flap Oligo.	CGCCGAGGATCGGGTTTTAG/3C6/
SP9	SP9_FP	Forward Primer	TAGCGTCGAATGGAAGTTCGA
	SP9_RP	Reverse Primer	GCGCGTAAACATAACGCACC
	SP9_Pb_A5	Flap Oligo.	CCACGGACGCCGTACGAATCC/3C6/
DMRTA2	DMRTA2_FP	Forward Primer	TGGTGTTCAGTTTCGGTTTTCTG
	DMRTA2_RP	Reverse Primer	CCGCAACAACGACGACC
	DMRTA2_Pb_A1	Flap Oligo.	CGCCGAGGCGAACGATCACG/3C6/
OPLAH	FPrimerOPLAH	Forward Primer	cGTcGcGTTTTTcGGTTATACG
	RPrimerOPLAH	Reverse Primer	CGCGAAAATAAAAAACCGCG
	ProbeA5OPLAH	Flap Oligo.	CCACGGACG-GCACCGTAAAC/3C6/
CYP26C1	CYP26C1_FP	Forward Primer	TGGTTTTTGGTTATTTCTGGAATCGT
	CYP26C1_RP	Reverse Primer	GCGCGTAATCAACGCTAAC
	CYP26C1_Pb_A1	Flap Oligo.	CGCCGAGGCGACGATCTAAC/3C6/
ZNF781	ZNF781F.primers	Forward Primer	CGTTTTTTGTTTTTCGAGTGCG
	ZNF781R.primers	Reverse Primer	TCAATAACTAACTACCGCGTC
	ZNF781probe.A5	Flap Oligo.	CCACGGACGGCGGATTTATCG/3C6/
DLX4	DLX4_FP	Forward Primer	TGAGTGCGTAGTGTTCGG
	DLX4_RP	Reverse Primer	CTCCTCTACTAAACGTACGATAAACA
	DLX4_Pb_A1	Flap Oligo.	CGCCGAGGATCGTATAAAC/3C6/
SUCLG2	SUCLG2_HM_FP	Forward Primer	TCGTGGGTTTTTAATCGTTTCG
	SUCLG2_HM_RP	Reverse Primer	TCACGCCATCTTACCGC
	SUCLG2_HM_Pb_A5	Flap Oligo.	CCACGGACGCGAAAATCTACA/3C6/
KLHDC7B	KLHDC7B_FP	Forward Primer	AGTTTTTCGGGTTTTGGAGTTCGTTA
	KLHDC7B_RP	Reverse Primer	CCAAATCCAACCGCCGC
	KLHDC7B_Pb_A1	Flap Oligo.	CGCCGAGGACGGCGGTAGTT/3C6/
S1PR4_HM	S1PR4_HM_FP	Forward Primer	TTATATAGGCGAGGTTGCGT
	S1PR4_HM_RP	Reverse Primer	CTTACGTATAAATAATACAACCACGAATA
	S1PR4_HM_Pb_A5	Flap Oligo.	CCACGGACGACGTACCAAACA/3C6/

NFIH_HM	NFIH_HM_FP	Forward Primer	TGGTTCGGGCGTGACGCG
	NFIH_HM_RP	Reverse Primer	TCTAACCTATTTAACCAACCGA
	NFIH_HM_Pb_A1	Flap Oligo.	CGCCGAGGGCGGTTAAAGTG/3C6/
Reference DNAs	Oligonucleotide Name	Component	Sequence (5'-3')
Zebrafish Synthetic (RASSF1) BT converted)†	ZF_RASSF1_FP	BT Forward Primer	TGCGTATGGTGGGCGAG
	ZF_RASSF1_RP	BT Reverse Primer	CCTAATTTACACGTCAACCAATCGAA
	ZF_RASSF1_Pb_A5	BT Flap Oligo.	CCACGGACGGCGCGTTCGTTT/3C6/
B3GALT6*	B3GALT6_FP_V2	Forward Primer	GGTTTATTTTGGTTTTTGGAGTTTTTCGG
	B3GALT6_RP	Reverse Primer	TCCAACCTACTATATTTACGCGAA
	B3GALT6_Pb_A1	Flap Oligo.	CCACGGACGGCGGATTAGGG/3C6/
BTACT	ACTB_BT_FP65	Forward Primer	GTGTTTGTTTTTTGGATTAGGTGTTTAAGA
	ACTB_BT_RP65	Reverse Primer	CTTTACACCAACCTCATAACCTTATC
	ACTBBTPbA3	Flap Oligo.	GACGCGGAGATAGTGTGTGG/3C6/

*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.

5 †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.

10

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	NFIH

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)
10	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)
15	PRKCB_28-SOBP-BTACT	(PSA2 Triplex)

Group 2:

	ZF_RASSF1-B3GALT6-BTACT	(ZBA Triplex)
	MAX.chr8.145-MAX_chr10.226-BTACT	(MMA2 Triplex)
20	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)
25	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 1×10^5 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 C_p 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%
FERMT3	0.773	36%

Marker Name	AUC	Sensitivity at 90% specificity
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).

10

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 methylated 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
5 (*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
10 in plasma achieved high sensitivity and specificity for all types of lung cancer.

EXAMPLE 5

Differentiating Lung Cancers

15 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
20 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
25 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.

30 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.

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
5 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.

10 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,
15 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 processing a sample, the method comprising:
 - a) assaying a sample from a subject for an amount of at least one methylation marker selected from the group consisting of *BARX1*, *LOC100129726*, *SPOCK2*, *TSC22D4*, *MAX.chr8.124*, *RASSF1*, *ZNF671*, *ST8SIA1*, *NKX6_2*, *FAM59B*, *DIDO1*, *MAX_Chr1.110*, *AGR1*, *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 *ZNF329*;
 - b) assaying said sample for an amount of a reference marker;
 - c) comparing the amount of said at least one 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 optionally
 - d) generating a record reporting the methylation state for said at least one methylation marker in said sample.

2. The method of claim 1, wherein said assaying comprises obtaining a sample comprising DNA from a subject and treating DNA obtained from the sample with a reagent that selectively modifies unmethylated cytosine residues in the obtained DNA to produce modified residues.

3. The method of claim 1 or claim 2, wherein said at least one methylation marker comprises a methylation 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*.

4. The method of any one claims 1 to 3, wherein said at least one methylation marker comprises at least two of said methylation markers, preferably 2 to 21 markers, preferably 2 to 8 markers, preferably 4 to 6 markers.
5. The method of claim any one of claims 1 to 4, wherein said at least one methylation marker comprises a group of methylation 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*.
6. The method of claim 5, wherein said at least one methylation marker comprises the group consisting of *ZNF781*, *BARX1*, and *EMX1*, and further comprises *SOBP* and/or *HOXA9*.
7. The method of claim 2, wherein said reagent comprises a bisulfite reagent.
8. The method of any one of claims 1 to 7, wherein assaying the methylation state of the methylation marker in the sample comprises determining the methylation state of one base.
9. The method of any one of claims 1 to 7, wherein assaying the methylation state of a methylation marker in the sample comprises determining the extent of methylation at a plurality of bases.
10. The method of any one of claims 1 to 9, wherein the methylation state of the methylation marker comprises increased or decreased methylation of the methylation marker relative to a methylation state of the same methylation marker in one or more normal samples, and/or wherein the methylation state of the methylation marker comprises a different pattern of methylation relative to a pattern of methylation of the same methylation marker in one or more normal samples.

11. The method of any one of claims 1 to 10, wherein said reference marker is a methylated reference marker.
12. The method of any one of claims 1 to 11, wherein the sample is a tissue sample, a blood sample, a serum sample, or a sputum sample.
13. The method of claim 12, wherein said tissue sample comprises lung tissue.
14. The method of any one of claims 1 to 12, wherein said sample is a plasma sample.
15. The method of any one of claims 1 to 12, wherein said sample is DNA isolated from plasma.
16. The method of any one of claims 1 to 15, wherein said subject has or is suspected of having a lung neoplasm.
17. A method of analyzing a plasma sample obtained from a subject suspected of having a lung neoplasm, the method comprising:
 - a) combining the plasma sample with:
 - i) protease; and
 - ii) a first lysis reagent, said first lysis reagent comprising
 - guanidine thiocyanate; and
 - non-ionic detergent;to form a mixture wherein proteins are digested by said protease;
 - b) to the mixture of step a) adding
 - iii) silica particles, and
 - iv) a second lysis reagent, said second lysis reagent comprising:
 - guanidine thiocyanate;
 - non-ionic detergent; and
 - isopropyl alcohol;under conditions wherein DNA is bound to said silica particles;

- c) separating silica particles with bound DNA from the mixture of b);
- d) to the separated silica particles with bound DNA adding a first wash solution, said first wash solution comprising A) guanidine hydrochloride or guanidine thiocyanate, and B) ethyl alcohol;
- e) separating the silica particles with bound DNA from said first wash solution;
- f) to the separated silica particles with bound DNA adding a second wash solution, said second wash solution comprising a buffer and ethyl alcohol;
- g) separating washed silica particles with bound DNA from said second wash solution; and
- h) eluting DNA from the washed silica particles with bound DNA separated in step g);
- i) assaying eluted DNA for an amount of at least one methylated methylation marker and for an amount of reference marker in said eluted DNA;
- j) comparing the amount of said at least one methylated methylation marker to the amount of reference marker in said DNA to determine a methylation state for said at least one methylation marker in said plasma sample; and optionally
- d) generating a record reporting the methylation state for said at least one methylation marker in said plasma sample.

18. The method of claim 17, wherein said at least one methylation 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*, *SLC12A8*, *BHLHE23*, *CAPN2*, *FGF14*, *FLJ34208*, *B3GALT6*, *BIN2_Z*, *DNMT3A*, *FERMT3*, *NFIX*, *S1PR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC*, and *ZNF329*.

19. The method of any one of claims 1 to 18, wherein the assaying comprises using polymerase chain reaction, nucleic acid sequencing, mass spectrometry, methylation specific nuclease, mass-based separation, or target capture.

20. The method of claim 19, wherein the assaying comprises using a flap endonuclease assay.
21. The method of any one of claims 1 to 20, wherein the assaying comprises bisulfite converting said marker DNA and said reference nucleic acid.
22. The method of claim 21, wherein determining the methylation level of one or more methylation markers is achieved by a technique selected from the group consisting of methylation-specific PCR, quantitative methylation-specific PCR, methylation-sensitive DNA restriction enzyme analysis, quantitative bisulfite pyrosequencing, flap endonuclease assay, and bisulfite genomic sequencing PCR.
23. The method of any one of claims 17 to 22, wherein said assaying comprises analyzing multiple DNA methylation markers using a PCR pre-amplification and a PCR-flap assay by a process comprising:
- a) providing bisulfite-treated DNA comprising a plurality of different DNA methylation marker target regions in a first reaction mixture comprising PCR amplification reagents, wherein said PCR amplification reagents comprise:
 - i) a plurality of different primer pairs for amplifying said plurality of different target regions, if present in said sample, from said bisulfite-treated DNA;
 - ii) thermostable DNA polymerase;
 - iii) dNTPs; and
 - iv) a buffer comprising Mg^{++}
 - b) exposing said first reaction mixture to thermal cycling conditions wherein a plurality of different DNA methylation marker target regions, if present in the sample, are amplified to produce a pre-amplified mixture, and wherein said thermal cycling conditions are limited to a number of thermal cycles that maintain amplification in an exponential range, preferably fewer than 20, more preferably fewer than 15, more preferably 10 or fewer thermal cycles;

- c) partitioning said pre-amplified mixture into a plurality of PCR-flap assay reaction mixtures, wherein each PCR-flap assay reaction mixture comprises:
 - i) an additional amount of a primer pair selected from said plurality of different primer pairs of step a) i);
 - ii) thermostable DNA polymerase;
 - iii) dNTPs;
 - iv) said buffer comprising Mg^{++}
 - v) a flap endonuclease;
 - vi) a flap oligonucleotide, and
 - vi) a hairpin oligonucleotide comprising a region that is complimentary to a portion of said flap oligonucleotide;

and

- d) detecting amplification of one or more different DNA methylation marker target regions from said bisulfite-treated DNA during PCR-flap assay reactions.

24. The method of claim 23, wherein said bisulfite-treated DNA is prepared from a plasma sample of at least one mL, preferably at least 5 mL, more preferably at least 10 mL, and wherein the volume of said bisulfite-treated DNA comprising a plurality of different DNA methylation marker target regions in the amplification reaction of step b is at least 20 to 50% of the total volume of the amplification reaction.

25. A kit, comprising:

- a) at least one oligonucleotide, wherein at least a portion of said oligonucleotide specifically hybridizes to a methylation 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*, and *ZNF329*, and

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

26. The kit of claim 25, wherein said portion of said oligonucleotide specifically hybridizes to a bisulfite-treated DNA comprising said methylation marker.

27. The kit of claim 25 or claim 26, wherein said kit comprises at least two additional oligonucleotides.

28. The kit of claim 25 or claim 26, wherein said kit further comprises bisulfite.

29. The kit of any one of claims 25 to 28, wherein at least a portion of said oligonucleotide specifically hybridizes to a methylation 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*.

30. The kit of any one of claims 25 to 29, wherein said at least one methylation marker comprises at least two of said methylation markers, preferably 2 to 21 markers, preferably 2 to 8 markers, preferably 4 to 6 markers.

31. The kit of any one of claims 25 to 30, wherein said at one methylation markers comprises a group of methylation markers selected from:

- the group consisting of *ZNF781*, *BARX1*, and *EMX1*;
- 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*.

32. The kit of claim 31, wherein said at least one methylation marker comprises the group consisting of *ZNF781*, *BARX1*, and *EMX1*, and further comprises *SOBP* and/or *HOXA9*.

33. The kit of any one of claims 25 to 32, wherein said 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 25 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 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 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*, *SIPR4*, *SKI*, *SUCLG2*, *TBX15*, *ZDHHC1*, and *ZNF329*, and an oligonucleotide that specifically hybridizes to said target nucleic acid.

39. The composition of claim 38, wherein said target nucleic acid is bisulfite-converted target nucleic acid.

40. The composition of claim 38 or claim 39, wherein said reaction 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 said target nucleic acid.

41. The composition of any one of claims 38 to 40, wherein said oligonucleotide is selected from 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.

42. The composition of any one of claims 38, 40 or 41, wherein said target nucleic acid 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.

43. The composition of any one of claims 39, 40 or 41, wherein said bisulfite-converted target nucleic acid 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, and 404.

44. The composition of any one of claims 38 to 43, wherein said oligonucleotide comprises a reporter molecule.

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

46. The composition of claim any one of claims 38 to 45, wherein said oligonucleotide comprises a flap sequence.

47. The composition of any one of claims 38 to 46, 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' GTTCCCGGAACGGCCTCTTTGGGGCGTTCCAGCCCCACGGACCCCGCAGGGAGTCCCCCGCCGCAATTTCATGGGG
CTCATTTGCATGACCCCGCCCGCGGGAGTCGGGGCGC3'

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

5' GTTTTCGGAACGGTTTTTTGGGGCGTTTAGTTTACGGATTCGTAGGAGTTTCGTCGTAATTGTATGGGG
TTTATTTGATGATTCGTTTCGCGCGGAGTCGGGGCGT3'

PCR and Flap Assay Oligonucleotides:

AGRN Forward Primer:	5' GCGGTTTAGTTTACGGATTCG3' (SEQ ID NO:3)
AGRN Reverse Primer:	5' ACAATAAACCCCATACAAATTACGAC3' (SEQ ID NO:4)
AGRN Flap oligo.:	5' CGCCGAGCGGAAACTCCCT/3C6/ (SEQ ID NO:5)

FIG. 1 (cont'd)

ANGPT1 Target DNA: (SEQ ID NO:6)

5' CGGATTCAACATGGGCAATGTGCTACACTTTCATTCTTCCAGAACACGATGGCAACTGTCGTGAGAGTACGACA
GACCAGTACAACACAACGCTCTGCAGAGAGATGCTCCACACGTGGAACCG3'

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

5' CGGATTAAATATGGGTAATGTGTTATATTTTATTTTATAGAAATACGATGGTAATTGTCGTGAGAGTACGATA
GATTAGTATAATATAAAACGTTTGTAGAGAGATGTTTATACGTGGAATCG3'

PCR and Flap Assay Oligonucleotides:

ANGPT1 Forward Primer: 5' TTTTAGAATACGATGGTAATTGTCGT3' (SEQ ID NO:8)

ANGPT1 Reverse Primer: 5' ACATCTCTCTACAAAACGTTTATATATATACTAATC3' (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' GGAGCTACGACGAGCAGCTGCGGCTGGCGATGGAACCTGTCGGCGCAGGAGCAGGAGAGGCGGCGCGCGC
GCCAGGAGGAGGAGGAGCTGGAGCGCATCCTGAG3'

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

5' GGAGTTACGACGAGTAGTTGCGGTTGGCGATGGAATTGTCGGCGTAGGAGTAGGAGAGGCGGCGCGCGC
GTTAGGAGGAGGAGGAGTTGGAGCGTATTTGAG3'

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' GGTGGCAACGGCTGGAGTGCCTCGCCCGGCCACTCACCCCGGGCGGCCCTGCGCGCCGCTCAGCGGAAG
GCCAGCAGGAAGATCAGTACGACGTTGATGAGAACAGGAGCGCCAGCACGGCGGAGACCACCGCG3'

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

5' GGTGGTAACGGTTGGAGTGTCTCGTTCGCGTTATTTCGGCGGCGGTTTTCGGCGGTCGTTAGCGGAAG
GTTAGTAGGAAGATTAGTACGACGTTGATGAGAAATTAGGAGCGTTAGTACGGCGGAGATTATTACGCG3'

PCR and Flap Assay Oligonucleotides:

ARHGEF4 Forward Primer:	5' CGTTCGCGTTATTATTTCGGCG3' (SEQ ID NO:18)
ARHGEF4 Reverse Primer:	5' GCTCCTAATTCTCATCAACGTCGT3' (SEQ ID NO:19)
ARHGEF4 Flap oligo.:	5' CGCCGAGGGCGGCGTTTTC/3C6/ (SEQ ID NO:20)

FIG. 1 (cont'd)

B3GALT6 Target DNA: (SEQ ID NO:384)

5' GGCCACACAGGCCCACTCTGGCCCTCTGAGCCCCCGGGACCCAGGGCATTC AAGGAGGGCTCTGGGCTGCCA
GCGAGGCCCTCCGCGCAACACACAGCAGGCTGGAAGTGGCGCTCATCACCGCACGCTCTCCACAG3'

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

5' GGTATATAGGTTATTTGGTTTTTGAGTTTTCGGCGATTAGGGTATTTAAGGAGCGTTTGGGTTGTTA
GCGTAGGTTTTCGCGTAAATATAGTAGGTTGGAAGTGGCGTTTATTATCGGTACGTTTTTTAG3'

PCR and Flap Assay oligonucleotides:

B3GALT6 Forward Primer: 5'GGTTATTTTGGTTTTTTGAGTTTTCGG3' (SEQ ID NO:386)

B3GALT6 Reverse Primer: 5'TCCAACCTACTATATTACGCGAA3' (SEQ ID NO:387)

B3GALT6 Flap oligo.: 5'CCACGGACGGCGGATTTAGGG/3C6/ (SEQ ID NO:388)

FIG. 1 (cont'd)

BARX1 Target DNA: (SEQ ID NO:21)

5' GGCCCGGGCCCTGGGCCCTAGGGCTGGACGTCAACCTGTTAGATAGAGGGCGTGGACCCCCCGCAGGCG
GCTGCTCGGACGACCGCATCCGGAG3'

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

5' GGTTCCGGGTCGTTTGGGTTTTAGGGTTGGACGTTAATTTGTTAGATAGAGGGCGTGGGATTTTCGTAGGCG
GTTGTTCCGACGATCGTATTCGGAG3'

PCR and Flap Assay Oligonucleotides:

BARX1 Forward Primer: 5' CGTTAATTGTTAGATAGAGGGCG3' (SEQ ID NO:23)

BARX1 Reverse Primer: 5' ACGATCGTCCGAACAACCC3' (SEQ ID NO:24)

BARX1 Flap oligo.: 5' CCACGGACGCGCCTACGAAA/3C6/ (SEQ ID NO:25)

BARX1 Forward Primer: 5' CGTTAATTGTTAGATAGAGGGCG3' (SEQ ID NO:23)

BARX1 Reverse Primer Universal: 5' TCCGAACAACCGCCTAC3' (SEQ ID NO:26)

BARX1 Flap oligo. Universal: 5' CCACGGACGCGAAAATCCCA/3C6/ (SEQ ID NO:27)

FIG. 1 (cont'd)

BCAT1 Target DNA: (SEQ ID NO:28)

5' GCTTCCAGCCGGCGCTCCGTGCCACTGCCGCTCTCTGCAGCCCCGGCTCCCCGCAGCCTCCCCATGGCCAGCCCC
GCTTCGCTCCGTGCGGCCCTTGCCCCGCCAGTACCTCGAACCC3'

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

5' GTTTTAGTCGCGGTTTCGTGTTATTGTCGTTTTTTGTAGTTTCGCGTTTTCGTAGTTTTTTATGGTAGTTC
GTTTCGTTTCGTGCGGTTTTTGTTCGTAGTATTTCGAATT3'

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' CGCCGAGGCGTTTTTCGTAG/3C6/ (SEQ ID NO:32)

FIG. 1 (cont'd)

BCL2L11 Target DNA: (SEQ ID NO:33)

5' GCCCGCCGCACGCCGCAATGCTCCGCGCTCCCCGCGGGTCCGGCGACTCAGACAGGGACCGGAAAAAGAACCCACG
CAGAAAGAAAGCCCTATTCTTGTCTGTCGTCTGTTCCCTGTGCAGCCTTGCAGCCTCGCCGCCCGCCCGCGT3'

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

5' GTTCGTCGTACGTCGTAATGTTTCGCGTTTTTCGCGGGTCCGGCGAATTAGATAGGGATCGGAAAAAGAAATTACG
TAGAAGAAAGTTTATTTTGTCTGTTTGTGTAGTTTGTAGTTTCGTCGTTTTCGCGT3'

PCR and Flap Assay Oligonucleotides:

BCL2L11 Forward Primer:	5' CGTAATGTTTCGCGTTTTTCG3' (SEQ ID NO:35)
BCL2L11 Reverse Primer:	5' ACTTCTCTACGTAATCTTTTCCGA3' (SEQ ID NO:36)
BCL2L11 Flap oligo.:	5' CGCCGAGGCGGGTCCGGC/3C6/ (SEQ ID NO:37)

FIG. 1 (cont'd)

BHLHE23 Target DNA (SEQ ID NO:38)

5' GCCGGGAGTCGAGAAAGCAAGTACTAGCGCTCCAGGACCGCGCGCCCGCGCGCCCGCGCCCTC
GGTCCAGAGC3'

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

5' GTCGGGAGTCGAGAAAGTAAATAGCGTTTATAGGATCGCGCGTCGTTTCGCGTCGTTTTC
GGTTAGAGT3'

PCR and Flap Assay Oligonucleotides:

BHLHE23_F Forward Primer: 5' AGTATTAGCGTTTATAGGATCGCG3' (SEQ ID NO:40)

BHLHE23_R 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' GCCGGGAGCCCGCACTTCCTCCTCGGGGGCCTCAGAAACACAGGGCGGGGCCAGGGCGGGCCCC
CAGG3'

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

5' GTCGGGAGTTCGTATTTTTTTTCGGGGTTTAGAAAATTATAGGCGCGGGTTAGGGCGGGGTTTT
TAGG3'

PCR and Flap Assay Oligonucleotides:

BIN2_HM Forward Primer:	5' TCGGGAGTTCGTATTTTTTTTCGG3' (SEQ ID NO:45)
BIN2_HM Reverse Primer:	5' AAAACCGCGCCCTAAC3' (SEQ ID NO:46)
BIN2_HM Flap oligo.:	5' CGCCGAGGCCCCCGGCCCTA/3C6/ (SEQ ID NO:47)

FIG. 1 (cont'd)

BIN2_Z Target DNA: (SEQ ID NO:48)

5'CGGGGCTACCCCTCAGGCAGCGCTCGCTCGAGGCCAGCTTCCGAGCTCCAACCCCTGCCCGAAACCTCGGCCTCA
CTG3'

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

5'CGGGGTTATTATTAGTAGCGTTCGTTGAGGTTAGTTTTCGAGTTTAAATTTTGTTCGAAATTCGGTTTAA
TTG3'

PCR and Flap Assay Oligonucleotides:

BIN2_Z Forward Primer: 5'GGGTTTATTTTAGTAGCGTTCG3' (SEQ ID NO:50)
BIN2_Z Reverse Primer: 5'CGAAATTTCGAACAAAAATTAAACTCGA3' (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' TGTCCCTGACACGATGGCCACACAGGCACAGTTTGTGGTGATGCCCAGGGGCCCGCGGGCCACGGTGGTCCAGTT
TAACTCGGGCCCCGCACTCCTGAAGTTCCGCGGGGAGGAGAGGGCGTCCCTTTCGCAGCTCGG3'

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

5' TGTTTGTATACGATGGTTATAGGTATAGTTTGTGGTGATTTAGGGGTTCCGCGGTTTACGGTGGTTAGTT
TATATTCGGGTTTCGTATTTTGAAGTTTCGCGGGGAGGAGAGGGCGTTTTCGTAGTTCGG3'

PCR and Flap Assay Oligonucleotides:

CAPN2 Forward Primer:	5' TGATGTTTAGGGGTTTCGGG3' (SEQ ID NO:55)
CAPN2 Reverse Primer:	5' CGAAACTTCAAAAATACGAAACCCGA3' (SEQ ID NO:56)
CAPN2 Flap oligo.:	5' CGCCGAGGCGGTTTACGG/3C6/ (SEQ ID NO:57)

FIG. 1 (cont'd)

chr5_132 Target DNA: (SEQ ID NO:58)

5' CCGGAGCACTCGCCGCTGCGGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAGGCCGGGGCTGGGCGGCTC
GGCAGCCTGCGCCGGGCTCCGCCCTCGGCCGCCAGC3'

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

5' TCGGAGTATTCGTCGTTGCGCGTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAGGTCGGCGGTTGGGCGGTTTC
GGTAGTTTCGTCGCGGTTTTCGTTTCGGTCGTAGT3'

PCR and Flap Assay Oligonucleotides:

chr5_132 Forward Primer: 5' GTATTCTGTCGTTGCGCG3' (SEQ ID NO:60)

chr5_132 Reverse Primer: 5' CCTCGAAACCGCCTACC3' (SEQ ID NO:61)

chr5_132 Flap oligo.: 5' CCACGGACGCGCCCAACGACTT/3C6/ (SEQ ID NO:62)

FIG. 1 (cont'd)

chr7_636 Target DNA: (SEQ ID NO:63)

5'CGCCGTGAGTGTTATAGTTCTTAAAGCGCGGTGTCCGGAGTTTCTTCCCTTCTGGTGGGTTTCGTGGTCTCGCCG
GCTCAGGAGTGAAGCTGCAGATCTTCGCGGTGAGTGTTACAGCTCCTAAGGCGGCAT3'

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

5'CGTCGTGAGTGTTATAGTTTAAAGCGCGGTGTTCGGAGTTTCTTCTTGGTGGGTTTCGTGGTTCGTCTCGTCTCG
GTTTAGGAGTGAAGTTGTAGATTTTCGCGGTGAGTGTTATAGTTTAAAGCGGCAT3'

PCR and Flap Assay Oligonucleotides:

chr7_636_HM Forward Primer:	5'TAAAGCGCGGTGTTCG3' (SEQ ID NO:65)
chr7_636_HM Reverse Primer:	5'CAACTTCACTCCTAAACCGAC3' (SEQ ID NO:66)
chr7_636_HM Flap oligo.:	5'CCACGGACGCGAAACCCACGAA/3C6/ (SEQ ID NO:67)

FIG. 1 (cont'd)

CYP26C1 Target DNA: (SEQ ID NO:68)

5'AACTGGCCCTTCTGGCTACTCCGGAAATCGCCAAAGCAGATGAGGCCAGACCCGCCGAGCGCTGATCACGCGCGCTC
CCACAGGTCCTGGCGCGCGTGTTTCAGCCGCGC3'

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

5'AATTGGTTTTTTGGTTATTTCCGGAATCGTTAAGTAGATGAGGTTAGATCGTCGTTAGCGTTGATTACGCGCGTTT
TTATAGGTTTTGGCGCGGTGTTTAGTCGCGT3'

PCR and Flap Assay Oligonucleotides:

CYP26C1 Forward Primer: 5'TGGTTTTTTGGTTATTTCCGGAATCGT3' (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)

DID01 Target DNA: (SEQ ID NO:73)

5' GGAGCGGCAGAGAGAGGCCAGGCGCCGAGGCCAGGCGCCCTCCCCGTCCCCC
GCTGCTCCCC3'

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

5' GGAGCGGTAGAGGAGGATTAGCGTCGAGGTTAGGCCTTTCGTTTTTTCGTTGTTTTTC
GTTGTTTTTT3'

PCR and Flap Assay Oligonucleotides:

Primer	Sequence	SEQ ID NO
DID01 Forward Primer:	5' GAGGAGGAGTTTAGCGTCG3'	(SEQ ID NO:75)
DID01 Reverse Primer:	5' CACGAAAAAACGAAAAACGAAAC3'	(SEQ ID NO:76)
DID01 Flap oligo.:	5' CGCCGAGGCGCGCCTAAACC/3C6/	(SEQ ID NO:77)

FIG. 1 (cont'd)

DLX4 Target DNA: (SEQ ID NO:78)

5' GCGGTCTATCACGGGACCCCTAACACTTGGTGAGTGCGCAGTGCTCTCGGCAGTCTCTGGGCTCCATACGATGC
CTACCGCACGCCCTAGCAGAGGAGGTCCTCTGT3'

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

5' GCGGTTTATTACGGGTAATTTTAATATTTGGTGAGTGCGTAGTGTTTTCGGTAGTTTTTGGGTTTATACGATGT
TTATCGTACGTTTAGTAGAGGAGGTTTTTGT3'

PCR and Flap Assay Oligonucleotides:

DLX4 Forward Primer:	5' TGAGTGCGTAGTGTTTCGG3' (SEQ ID NO:80)
DLX4 Reverse Primer:	5' CTCCTCTACTAAACGACGATACGATAAACCA3' (SEQ ID NO:81)
DLX4 Flap oligo.:	5' CGCCGAGGATCGTATAAAC/3C6/ (SEQ ID NO:82)
DLX4 Forward Primer Universal:	5' ATATTTGGTGAGTGCGTAGTG3' (SEQ ID NO:83)
DLX4 Reverse Primer Universal:	5' ACGTACGATAAACATCGTATAAACCA3' (SEQ ID NO:84)
DLX4 Flap oligo. Universal:	5' CGCCGAGGTTTTCGGTAGT/3C6/ (SEQ ID NO:85)

FIG. 1 (cont'd)

DMRTA2 Target DNA: (SEQ ID NO:86)

5' TACTCCACTGCCGGCTTGGTGCCACGCTCGGCTCCGCCACCCATGGACTACGCCCTTAGCGATCTCATGCCGT
GACCGTCGGCCGCGCTGCTGCGGGGTGCACAAGAGCCGACCT3'

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

5' TATTTTATTGTCGGTTGGTGTTACGTTCCGTTTTCGTTTATTATGGATTACGTTTTTAGCGATTTATGCCGT
GATCGTTCGGTCGTCGTTGTTGCGGGGTGTATAAGGAGTCGATT3'

PCR and Flap Assay Oligonucleotides:

DMRTA2 Forward Primer: 5' TGGTGTTACGTTCCGGTTTTCGT3' (SEQ ID NO:88)

DMRTA2 Reverse Primer: 5' CCGCAACAACGACGACC3' (SEQ ID NO:89)

DMRTA2 Flap oligo.: 5' CGCCGAGGCCGAACGATCACG/3C6/ (SEQ ID NO:90)

FIG. 1 (cont'd)

DNMT3A Target DNA: (SEQ ID NO:91)

5'AGGCCGGTCACGAACAAAGCGCTGGCCAGTGGCGGCCCGCCACGCGCACAGGTGCCCGCGACAAAGACGCCCGGT
CCCCGCCACGCGGCCCGCCCGGGCTGAGCC3'

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

5'AGGTCGGTTACGAATAAAGCGTTGGCGAGTGCGGGTCGTTACGCCGTATAGGTGTTCCGCATAAGACGTTTCGT
TTTCGTTTACGCGGTTTTCGCGGTTGAGTT3'

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' GCCGGCCCCGAGCATCCTCCTGCTCGCGGCTCTCCCGCCACCTGTCCCGCTCCCTGCCGCGCCCTGGGGCCCCGC
ACCTACCCAC3'

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

5' GTCGGTTCGTAGTATTTTGTTCGCGGTTTTTTCGTTATTGTTCTGTTTTTGTCCGCTTTTGGGTTTCGT
ATTATTAT3'

PCR and Flap Assay Oligonucleotides:

DOCK2 Forward Primer: 5' CGGTTTCGTAGTATTTTGTTCG3' (SEQ ID NO:98)

DOCK2 Reverse Primer: 5' GAACCCCAAAACGCGAC3' (SEQ ID NO:99)

DOCK2 Flap oligo.: 5' CGCCGAGGCGGTTTTTTCG/3C6/ (SEQ ID NO:100)

FIG. 1 (cont'd)

DTX1 Target DNA: (SEQ ID NO:101)

5' CGCCTCCTGGGCTCCCCCGGAGTGGGAGGAGCCGGGTCCCGCCTCCGGCCCGTTCCCTCCCAGGCCCTCG
GCCGCCGCCGAGCTTCCGCGCGTGGACAGACTGCCCGGCCGACGGACGACGACGG3'

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

5' CGTTTTTGGGTTTTTTCGGAGTGGGAGGAGTCGCGGTTTCGTTTTTCGCGTTCGTTTTTTAGGTTTTTCG
GTCGTCGCGTCGAGTTTTTCGCGCGTGGATAGATTGTTTCGTCGACGGACGACGTAGG3'

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'TCCGGCGCCGCGTTTCTAGAGAAACCGGGTCTCAGCGATGCTCATTTTCAGCCCCCGTCTTAATGCAACAAACGAAA
CCCCACACGAACGAAAGGAACATGCTGCGCT3'

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

5'TCGGCGTCGCGTTTCTAGAGAAATCGGGTTTTCAGCGATGTTATTTAGTTTCGTTTAAATGTAATAACGAAAT
TTTATACGAACGAAAGGAATATGTTGCGTT3'

PCR and Flap Assay Oligonucleotides:

EMX1 Forward Primer: 5'GGCGTCGCGTTTTTTAGAGAA3' (SEQ ID NO:108)

EMX1 Reverse Primer: 5'TTCCCTTTTCGTTTCGTATATAAAATTCGTT3' (SEQ ID NO:109)

EMX1 Flap oligo.:5'CCACGGACGATCGGGTTTAG/3C6/ (SEQ ID NO:110)

FIG. 1 (cont'd)

FAM59B Target DNA: (SEQ ID NO:111)

5' GGGCCTGCTGGCCGGGACCCGGCGTCGAGCGCCTGGTGCGGACAGCGCCTCCTACTGCCGCGAGCGCTTCGA
CCCCGACGAGTACTCCACGGCCGTGCGGAGGCCAGCGGAGCTCGCCGAAG3'

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

5' GGGTTTGTGGTCGGGATTCCGCCGTCGAGCGTTTGGTGCGGATAGCGTTTATTATTCGCGAGCGTTTCGA
TTTCGACGAGTATTTACGGTCGTGCGGAGGCGTTAGCGGAGTTCGTGGAAG3'

PCR and Flap Assay Oligonucleotides:

FAM59B Forward Primer: 5' CGATAGCGTTTTTTATTGTGCGG3' (SEQ ID NO:113)

FAM59B Reverse Primer: 5' GCACGACCGTAAATACTCGTC3' (SEQ ID NO:114)

FAM59B Flap oligo.: 5' CCACGGACGCGAAATCGAAAC/3C6/ (SEQ ID NO:115)

FIG. 1 (cont'd)

FERMT3 Target DNA: (SEQ ID NO:116)

5' TAGCAGCAGCCGCAGCCATGGCGGGGATGAAGACAGCCCTCCGGGGACTACATCGACTCGTCATGGGAGCTGCGGG
TGTTTGTGGAGAGGAGGCCACAGAGGCCGAGTCGGTCACCCCTGCGGGTCACTGGGGAGTCGCAC3'

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

5' TAGTAGTAGTCGTAGTTATGGCGGGGATGAAGATAGTTTTCGGGGATTATATCGATTCTGTTATGGGAGTTGCGGG
TGTTTGTGGAGAGGAGGATTAGAGGTCGAGTCGGTTATTTGCGGGTTATTGGGGAGTCGTAT3'

PCR and Flap Assay Oligonucleotides:

FERMT3 Forward Primer:	5' GTTTTCGGGGATTATATCGATTTCG3' (SEQ ID NO:118)
FERMT3 Reverse Primer:	5' CCCAATAACCCGCAAAATAACC3' (SEQ ID NO:119)
FERMT3 Flap oligo.:	5' CGCCGAGGCGGACTCGACCTC/3C6/ (SEQ ID NO:120)

FIG. 1 (cont'd)

FGF14 Target DNA: (SEQ ID NO:121)

5' GTCCCAGAGACGCCCTAGGGTCAGAGGTCACTCCGTGGCAACGGAAACTTCCCCGGCTACGGCGGCTCCAAACGG
GCCGCTCCGCCGCATTGCGTAGCGAAGC3'

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

5' GTTTTAGAGACGTTTtaggTtagagTTATTTCGTGGTAACGGAAATTTTCGGTTACGGCGGTTTAAACGG
GTCGTTTTCGTCGATTGCGTAGCGAAGT3'

PCR and Flap Assay Oligonucleotides:

FGF14 Forward Primer: 5' TTTCGTGGTAACGGAAATTTTCG3' (SEQ ID NO:123)

FGF14 Reverse Primer: 5' CGACGAAACGACCCGT3' (SEQ ID NO:124)

FGF14 Flap oligo.: 5' CGCCGAGGCGTTACGGCGG/3C6/ (SEQ ID NO:125)

FIG. 1 (cont'd)

FLJ34208 Target DNA: (SEQ ID NO:126)

5' GCGCCCCGGCCGAGGCGGAGGACAGGAGGAGCGGCACACGAGAAAGCTCCACGGCCCCGGCCTCGCCTCCGA
CGGGAAGGCGCCTCTTCCGACCGTCCTGGATG3'

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

5' GCGTTTCGGTCGTAGGCGGAGGATAGGAGGAGCGGTATACGAGAAAGTTTACGGTTCGGTTTCGTTTCGA
CGGGAAGGCGTTTTTTCGATCGTTTGGATG3'

PCR and Flap Assay Oligonucleotides:

FLJ34208 Forward Primer:	5' GAGCGTATACGAGAAAGTTTACG (SEQ ID NO:128)
FLJ34208 Reverse Primer:	5' AACGCCCTTCCCGTCGAA (SEQ ID NO:129)
FLJ34208_Flap oligo.:	5' CCACGGACGGCGTTTCGCGTTT/3C6/ (SEQ ID NO:130)

FIG. 1 (cont'd)

FLJ45983 Target DNA: (SEQ ID NO:131)

5' CGAGAGGGCGGAGCACAGCCGAGGCCATGGAGGTGACGGCGGACCAGCCGCGCTGGGTGAGCCACCACACCCC
GCCGTGCTCAACGGGCGAGCACCCGGACACGCAC3'

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

5' CGAGAGGGCGGAGTATAGTCGAGGTATGGAGGTGACGGCGGATTAGTCGCGTTGGGTGAGTTATTATTTC
GTCGTGTTAACGGGTAGTATTCGGATACGTAT3'

PCR and Flap Assay Oligonucleotides:

FLJ45983 Forward Primer: 5' GGGCGCGAGTATAGTCG3' (SEQ ID NO:133)

FLJ45983 Reverse Primer: 5' CAACGGGACTAATCCGC3' (SEQ ID NO:134)

FLJ45983 Flap oligo.: 5' CGCCGAGGCGGTCACTCCA/3C6/ (SEQ ID NO:135)

FIG. 1 (cont'd)

GRIN2D Target DNA: (SEQ ID NO:136)

5' CGCCCCCTCACCTCCCCGATCATGCCGTTCCAGACGCCATCGATCTTCTTCCGTGCTTGCCATTGGTGACCAGG
TAGAGGTCGTAGCTGAAGCCGATGGTATGCGCCAGCCGCTTCAGAAATGTCGATGCAGAAACCCCTTG3'

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

5' CGTTTTTTTATTTTTCGATTATGTCGTTTTAGACGTTATCGATTTTTTTTCGTGTTTGTATTGGTGATTAGG
TAGAGGTCGTAGTTGAAGTCGATGGTATGCGTTAGTCGTTTGAAGTGTGATGAGAAATTTTG3'

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' CCACGGACGGCATACCATCG/3C6/ (SEQ ID NO:140)

FIG. 1 (cont'd)

HIST1H2BE Target DNA: (SEQ ID NO:141)

5' CGGCGAGGCTTCCCCGCTGGCGCATTAACAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCG
CCTGCTGCTTCCCCGGGA3'

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

5' CGGCGAGGTTTTCGTTTGGCGTATTATAAAGCGTTCGATTATTATTTAGGGAGATTAGACGGTCGTGCG
TTTGTGTTTTCGGGA3'

PCR and Flap Assay Oligonucleotides:

HIST1H2BE Forward Primer: 5' TGGCGTATTATAATAAGCGTTCG3' (SEQ ID NO:143)

HIST1H2BE Reverse Primer: 5' AACAAACAAACGCACGACC3' (SEQ ID NO:144)

HIST1H2BE Flap oligo.: 5' CCACGGACGGCTCTAAATCTC/3C6/ (SEQ ID NO:145)

FIG. 1 (cont'd)

HOXA9 Target DNA: (SEQ ID NO:146)

5' GGGCGGGCCAGGCGCTGGGCACGGTGATGGCCACCACCTGGGGCCCTGGGCAACTACTACGTGGACTCGTTCCTGC
TGGCGCCGACGCCGGGATGAGCTG3'

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

5' GGGCGGGTTAGGCGTTGGGTACGGTGATGGTTATTATTGGGGTTTGGGTAATTATTACGTGGATTCTTTTGT
TGGGCGTCGACGTCGCGGATGAGTTG3'

PCR and Flap Assay Oligonucleotides:

HOXA9 Forward Primer: 5' TTGGGTAATTATTACGTGGATTG3' (SEQ ID NO:148)

HOXA9 Reverse Primer: 5' ACTCATCCGCGACGTC3' (SEQ ID NO:149)

HOXA9 Flap oligo.: 5' CCACGGACGCGACGCCCAACA/3C6/ (SEQ ID NO:150)

FIG. 1 (cont'd)

HOXB2 Target DNA: (SEQ ID NO:151)

5' GGGCCATTGCCAGAGACGTCTTCTCGGGGCGCCAGGATTCAACCTTCCCTTCCCGACCTCAACTTCTTCGCGGCC
GACTCCTGTCTCCAGCTATC3'

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

5' GGGTTATTGTTAGAGACGTTTTTTCGGGGCGTTAGGATTATTTTTTTTTTCGATTTTAAATTTTTTCGCGGTC
GATTTTGTTTTTAGTTATT3'

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' CGCCGAGGGCGTTAGGATT/3C6/ (SEQ ID NO:155)

FIG. 1 (cont'd)

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

5' GGCCCCGGGAAGCCAGCTCCCCGGGCCCTGGAGCCCCGCCACGGCGGCA GCCCTGCGGGCGGGCTGGACCTGGGCA
GTTGCCCTGGACGTGCTGGCCCTTTGCCCCAGCA3'

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

5' GGTTTCGGAAGTTAGTTTTCGGGTTTTCGGAGTCGTTACGGCGGTAGTTTTCGGCGCGGTTGGATTGGGTA
GTTGTTGGACGTGTTGGTTTTCGTTAGTA3'

PCR and Flap Assay Oligonucleotides:

KLHDC7B Forward Primer:	5' AGTTTTCGGGTTTTCGGAGTTCGTTA3' (SEQ ID NO:158)
KLHDC7B Reverse Primer:	5' CCAATCCAAACCGCCGC3' (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' GGCGGCGCCGGCGCTGCCGGGGGCACGCCCTGCTGCTGCTGCTGCTGCTAGTAGGCGCG
GCGGCCACGGCGCAAGTTGTGGTCTGGA

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

[illegible]

PCR and Flap Assay Oligonucleotides:

LOC100129726 Forward Primer: 5'TTGATTGCGTAGGCG3' (SEQ ID NO:163)

LOC100129726 Reverse Primer: 5'AACCCACAACCTTACCGCC3' (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'GGTTTCCCCACCCCGGCTCGGGTCTCTCCACGTCTCCCCGCCGACGTGCTCACCTGCTCAGGGGGCCCC
CGAGCCGGCCCCCGCCCCCAGGAGGGCTCCGCCGAGCCGGCTGCACACCCGAGGGGTCCCCGGCTGCACA
AC3'

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

5'GGTTTTTTTATTTCGGTTTCGGGTTTTTTACGTTTTTCGTCGACGTATTATTGTTAGGGGCGTTTT
CGAGTCGGTTTCGGTTTCGTTTTTAGGAGGTTTTTCGCGAGTCGGTTGTATATTTCGAGGCGTTTCGGTTGTATA
AT3'

PCR and Flap Assay Oligonucleotides:

MATK Forward Primer: 5'GTTTCGGGGTTTTTTACGTTTTTTTCG3' (SEQ ID NO:168)

MATK Reverse Primer: 5'AAACGCGACTCGAAAACGC3' (SEQ ID NO:169)

MATK Flap oligo.: 5'CGCCGAGGTCGACGTGTTT/3C6/ (SEQ ID NO:170)

FIG. 1 (cont'd)

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

5'CTCCGGTTTTCGCGGTTCTCAGCGATATTAGGCGGCGCCAGTGCTGAAAGCTCCTCGGGGTTACGTCCTGGGGC
GACTGGAGGCGGCTCACGAC3'

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

5'TTTCGGTTTTCGCGGTTTTCAGCGATATTAGGCGCGGTTAGTGTTTGAAGTTTTCGGGGTACGTTTGGGGC
GATTGGAGGCGGTTACGAT3'

PCR and Flap Assay Oligonucleotides:

MAX_chr10.225 Forward Primer: 5'CGGTTTTCAGCGATATTAGCG3' (SEQ ID NO:173)

MAX_chr10.225 Reverse Primer: 5'CCCAAAACGTAAACCCCA3' (SEQ ID NO:174)

MAX_chr10.225 Flap oligo.: 5'CGCCGAGGCGGTTAGTGTT/3C6/ (SEQ ID NO:175)

FIG. 1 (cont'd)

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

5' CGACGGCCGCGAGGAGGAGGCCAGGGGAAATTTCATTTTCGTAAACCGCGGTTAAGAAATGACGATGCCAC
GTAGACAAGCCAGTTGTGACGTTCAGCACAAACGTGCTACTGAACACCGAGATCCGCCACCAAAATGGC3'

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

5' CGACGGTCGCGAGGAGGAAAGTTAGGGGAAATTTCATTTTCGTAAATCGCGGTTAAGAAATGACGATGTTAC
GTAGATAAGTTAGTTGTGACGTTTAGTATAACGTGTTATTGAATTATCGAGATTCGTTATTAAATGGT3'

PCR and Flap Assay Oligonucleotides:

MAX_Chr10.226 Forward Primer: 5'GGGAAATTGTATTTTCGTAAATCG3' (SEQ ID NO:178)
MAX_Chr10.226 Reverse Primer: 5'ACAACTAACTTATCTACGTAAACATCGT3' (SEQ ID NO:179)
MAX_Chr10.226 Flap oligo.: 5'CCACGGACGCGGTTAAGAAA/3C6/ (SEQ ID NO:180)

FIG. 1 (cont'd)

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

5' GGCTTGGGGTCCAGCCGCCCGCCCTGCCGCCACCGACCATGTCTGCTCTACTCCCCGCTCAGCGCCCCCTG
CGGGTCCGGCCTTCAGCTGCATCTCGGCCTGCGGGCCCCC3'

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

5' GGTTGGGGTTAGTCGTTTCGTTTTTGTCTATCGTATTATGTTTGTATTTCGTTTAGCGTTTTTG
CGGGTCCGGTTTTAGTTGATTTTCGGTTGCGGGTTT3'

PCR and Flap Assay Oligonucleotides:

MAX.chr12.52 Forward Primer: 5' TCGTTCGTTTTTGTCTATCG3' (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' GGAAGGCTGCAGCGAGAGATTTACATATTTCATCCGAGCTTAAGGAAAGCCGCGATAATGCAGGTACAGCCCCGAAAC
CCACGCCCCCAGACCTTATCTGCGCGCCCCGCC3'

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

5' GGAAGGTTGTAGCGAGAGATTATATATTATTCGAGTTTAAGGAAGTCGCGATAATGTAGGTATAGTTCGAAAT
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'TCTAAAAACGTAAATTTTCGAACT3' (SEQ ID NO:189)

MAX.chr16.50 Flap oligo.: 5'CCACGGACGCGGATAATGTAG/3C6/ (SEQ ID NO:190)

FIG. 1 (cont'd)

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

5' GGAGTTATTTTAACCATCGCCCTCCCAGAACATTACGGAGCTTCCTCTCTCCAACACGACGAGAAACCCCTACTTGG
CTGTGCTTCCTGCTAACACGAGGCCCTGCGATTGCTGAGAACACAGCCCCGAGACTGCGCGG3'

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

5' GGAGTTATTTTAATTATCGTTTTTTAGAAATATACGGAGTTTTTTTTTTTAATACGTAGGAAATTTATTGG
TTGTGTTTTTGTAAATACGAGGTTTTTGGGATTGTTGAGAAATAAGTTTCGAGATTGCGCGG3'

PCR and Flap Assay Oligonucleotides:

MAX.chr19.16 For. Primer: 5' TTTAATTATCGTTTTTTAGAAATATTACGGA3' (SEQ ID NO:193)

MAX.chr19.16 Reverse Primer: 5' ACTATTATTCTCAACAATCGCAAAAC3' (SEQ ID NO:194)

MAX.chr19.16 Flap oligo.: 5' CCACGGACGCCCTCGTATTAAAC/3C6/ (SEQ ID NO:195)

FIG. 1 (cont'd)

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

5' GCGGGCGCTTGGCCAAACAGCCCAAGACTGCGGGAATCACACTCGCCACTGTGTACCTGGACGCCATCTGCAGAC
CCAGCGCCTGCGGGGATTCCGGAAACGGGAGAGCGGGCTTCC3'

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

5' GCGGGCGCTTGGTTAAATAGTTAAGATTGCGGAAATATATTCGTTATTGTGATTGGACGTTATTGTAGAT
TTAGCGTTTGC GGGAATTCGGAAACGGGAGAGCGGGTTTTT3'

PCR and Flap Assay Oligonucleotides:

MAX.chr19.37 For. Primer_v2: 5' AGTTAAGATTGCGGAATTATATTCGT3' (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
GGCTGCAGGAGCCTCCCTGCGGGGCCCACTCCCTCATCTGCGACCCCGTGGGAGGCGACCCCTGACCAACCCCTCGT
TCCG3'

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

5' CGTAGGTTGAGGTTTTCGGGTTTTCAGCGGGTTTCGTTATTAGTTATTTTACGGGTTAGGTTGGGGGTAC
GGTTGTAGGAGTTTTTTGCGGGGTTTATTTTATTTGCGATTTCGTGGGAGGCGATTGTGATTATTTTCGT
TTCG3'

PCR and Flap Assay Oligonucleotides:

MAX_chr8.124 Forward Primer: 5'GGTTGAGGTTTTCGGGTTTTCGTTAG3' (SEQ ID NO:203)

MAX_chr8.124 Reverse Primer: 5'CCTCCCCACGAAATCGC3' (SEQ ID NO:204)

MAX_chr8.124 Flap oligo.: 5'CGCCGAGGGCGGTTTTCGT/3C6/ (SEQ ID NO:205)

MAX_chr8.124 Forward Primer v2: 5'AGGAGTTTTCGCGCGG3' (SEQ ID NO:206)

MAX_chr8.124 Reverse Primer v2: 5'ACGAAATAATCAAAATCGCCTCC3' (SEQ ID NO:207)

MAX_chr8.124 Flap oligo. v2: 5'CGCCGAGGCCACGAAATCG/3C6/ (SEQ ID NO:208)

FIG. 1 (cont'd)

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

5' CGGGGAGGGCGGCATCAGCCAGAGCCTCAGCCGACGGCGCTCCCCAGGTCCTCCCGCTCCGATACCCCTCCC
CCTAAGCACGATACCCAGGGCCAGGGCTGCTCTTGGCG3'

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

5' CGGGGAGGGCGGTATTAGTTAGATTTAGTCGACGGCGTTTATTAGGTTATTTTCGTTTCGATATTTTT
TTTAAGTACGATATTAGGGTTAGGGTGTGTTTGGCG3'

PCR and Flap Assay Oligonucleotides:

MAX_chr8.145 Forward Primer: 5' GCGGTATTAGTTAGAGTTTATAGTCG3' (SEQ ID NO:211)

MAX_chr8.145 Reverse Primer: 5' ACAACCCCTAAACCCCTAAATATCGT3' (SEQ ID NO:212)

MAX_chr8.145 Flap oligo.: 5' CCACGGACGGACGGCGTTT/3C6/ (SEQ ID NO:213)

FIG. 1 (cont'd)

MAX_chr1.110 (MAX.chr1.110627198-110627213) Target DNA: (SEQ ID NO:214)
 5'CTCCGCTCCCCGCAGGCCCTGGCCGCGACGGGCACCCAGCGGGTTGTTATCAATTATTCAGGCCCCAAAGTTCAC
 GGGCACTGCATCCATTCCCTCGCGTGCGCCCC3'

Bisulfite-converted Target DNA: (SEQ ID NO:215)
 5'TTTCGTTTTTCGTAGGTTTGGTCGCGACGGGTATTAGCGGGTTGTTATTAATTATTAGGTTTAAAGTTTAC
 GGGTATTGTATTATTTTTTCGCGTGCGTTT3'

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)

FIG. 1 (cont'd)

NFIX Target DNA: (SEQ ID NO:219)

5' GTGGGCGGCGGTGACGCGCGGTCAAAGTGCAATGATTTTCAGTTCGGTTGGCTAAACAGGGTCAGAGCTGAGA
GCGAAGCAGAAAGG3'

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

5' GTGGGTCGGCGGTGACGCGCGGTAAAGTGTAATGATTTTTCAGTTCGGTTGGTTAAATAGGGTTAGAGTTGAGA
GCGAAGTAGAAAGG3'

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' CGCCGAGGCGGTTAAAGTG/3C6/ (SEQ ID NO:223)

FIG. 1 (cont'd)

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

5' GGACCTCCTCGGCCCCCGCCCATCCGCCCTTCGGGATGCTGCTGAGCCCCGTCACTCCACCCCTTCTCGGTCAA
GGACATCCTGCGACTGGAG3'

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

5' GGATTTTTTCGGTTTCGTTTATTTCGTTTTCGGGATGTTGTTGAGTTTCGTTATTTTATTTTTCGGTTAA
GGATATTTTTCGATTTGGAG3'

PCR and Flap Assay Oligonucleotides:

NKX2-6 Forward Primer:	5' GATTTTTTCGGTTTCGTTTATTCG3' (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' CTGTCAGTGCTGACCGAGCGCGCCCTTCCGGCCATACGGGCTCCACGGTGCGCGGTTCCCCAGCCCTCGCGGC
CCTCCCCGCCCCCG3'

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

5' TTGTTAGTTGATCGAGCGTCGCGTTTTCGGTTATACGGGTTTACGGTGCGCGGTTTTTAGTTTTCGCGGT
TTTTTCGTTTTCG3'

PCR and Flap Assay Oligonucleotides:

OPLAH Forward Primer: 5' CGTCGCGTTCGCGTTATACG3' (SEQ ID NO:231)

OPLAH Reverse Primer: 5' CGCGAAAACATAAAAAACCGCG3' (SEQ ID NO:232)

OPLAH Flap oligo.: 5' CCACGGACGGCACCCGTAAAC/3C6/ (SEQ ID NO:233)

FIG. 1 (cont'd)

PARP15 Target DNA: (SEQ ID NO:234)

5' CGGAGTATGGTGAGGAGCGCGGGGACGGGTGCCGGAAGGGGACAGCAGGGCTGAGCCTGGGGCCCCGCAAGACCC
AGCAGCCCGAGCGGGCGCAGAGACCCACGCCACGCACA3'

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

5' CGGAGTATGGTGAGGAGCGCGGGGACGGGTGCCGGAAGGGGATAGTAGGTTGAGTTGGGGTTCGTAAGATT
AGTAGTCGAGCGGCGTAGAGATTTACGTTACGTATA3'

PCR and Flap Assay Oligonucleotides:

PARP15 Forward Primer:	5' GGTGAGTTTGGGGTTCG3' (SEQ ID NO:236)
PARP15 Reverse Primer:	5' CGTAACGTAAAATCTCTACGCCC3' (SEQ ID NO:237)
PARP15 Flap oligo.:	5' CCACGGACGGCTCGAACTAC/3C6/ (SEQ ID NO:238)

FIG. 1 (cont'd)

PRDM14 Target DNA: (SEQ ID NO:239)

5' GGAGAGCAGCCCGCAGAACCTGGCCGCGTACTACACGCCCTTCCCGTCCTATGGACACTACAGAAACAGCCTGGC
CACCGTGGAGGAAGACTTCC3'

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

5' GGAGAGTAGTTCGTAGAATTGGTCGCGTATTATACGTTTTTTTCGTTTATGGATATTATAGAAATAGTTTGGT
TATCGTGGAGGAAGATTTT3'

PCR and Flap Assay Oligonucleotides:

PRDM14 Forward Primer: 5' GAGTAGTTCGTAGAATTGGTCG3' (SEQ ID NO:241)
PRDM14 Reverse Primer v2: 5' CCACGATAACCAAACTATTTCTATAAATATCC3' (SEQ ID NO:242)
PRDM14 Flap oligo.: 5' CCACGGACGGCGTATTATACG/3C6/ (SEQ ID NO:243)

PRDM14 Forward Primer v3: 5' GGAGAGTAGTTCGTAGAATTGG3' (SEQ ID NO:244)
PRDM14 Rev. Primer v3: 5' CTATTTCTATAAATATCCATAAAACGAAAAAACGT3' (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' GGGAAAGGTGCCCTGCCGCGCGGCTCACCAGATGAAAGTCGGTGCAGTGGCTGCAGAAAGTGGGCTGCTTGAAGA
AGCGGGCGGTGAATTTG3'

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

5' GGGAAAGTGTTTGC GCGCGCGGTTATTAGATGAAAGTCGGTGTAGTGGTTGTAGAAGTGGGTTGTTGAAGA
AGCGGGCGGTGAATTTG3'

PCR and Flap Assay Oligonucleotides:

PRKCB_28 Forward Primer: 5' GGAAGGTGTTTGCGCG3' (SEQ ID NO:249)

PRKCB_28 Reverse Primer: 5' CTTCTACAACCACTACACCGA3' (SEQ ID NO:250)

PRKCB_28 Flap oligo.: 5' CCACGGACGCGCGGTTTAT/3C6/ (SEQ ID NO:251)

FIG. 1 (cont'd)

PTGDR Target DNA: (SEQ ID NO:252)

5' GCCTCGGGGCCCGGACTCACAAATTACGGGCAGAGAACACATAGTGAAGAGCACGGTCATCAGCGCCAGCAGCA
GGAGGTGATCCAGCTCCTCCAGGGGCTGAGGG3'

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

5' GTTTCGGGGTTCGGGGATTATAATTACGGGTAGAGAAATATATAGTGAAGAGTACGGTTATTAGCGTTAGTAGTA
GGAGGTGATTTAGTTTTTTAGGGGTTGAGGG3'

PCR and Flap Assay Oligonucleotides:

PTGDR Forward Primer:	5' GGGTTCGGGGATTATAATTACGG3' (SEQ ID NO:254)
PTGDR Reverse Primer:	5' CCTCCTACTACTAACGCTAATAACC3' (SEQ ID NO:255)
PTGDR Flap oligo.:	5' CCACGGACGCGTACTCTTCAC/3C6/ (SEQ ID NO:256)

FIG. 1 (cont'd)

PTGDR_9 Target DNA: (SEQ ID NO:257)

5' GGCGGCTGCAGCGGCACCCGGCTCCTGCACCAGGGACTGTGCCGAGCCGCGGACGGGAGGGAAGCGTCCC
CTCAG

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

5' GGCGGTTGTAGCGGTATTCGCGTTTTTGTATTAGGATTGTGTGAGTCGCGCGGACGGGAGGAGCGTTTT
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' CGCCGAGGCGCGGACTCGACA/3C6/ (SEQ ID NO:261)

FIG. 1 (cont'd)

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

5' TCCAGAAACACGGGTATCTCCGCGTGGTGCTTTGCGGTGCGCGTCGTTGTGGCCGTCCGCGGGTGGGGTGTGAGGA
GGGACGAGGAGGAGGAAAGGCAAGCGGGGGGGCTCTGCGAGAGCGGCCAGCCCCGCCCTTC3'

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

5' TTTAGAAATACGGGTATTTTCGCGTGGTGTTTTCGCGTCGTCGTCGTTGTGGTCTGTTCCGCGGTGGGGTGTGAGGA
GGGACGAGGAGGAGGAAAGGTAAGCGGGGGGGTTTTCGAGAGCGCGTTTAGTTTCGTTTTT3'

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' CCACGGACGGCAAAACACCA/3C6/ (SEQ ID NO:266)

FIG. 1 (cont'd)

SHOX2 Target DNA: (SEQ ID NO:267)

5' CCGTCGGGCAGCGGGACGGAGATTACCTGGCTGTCCAGGGGACCTTATGCAGGGTTTGGCCCCGAGCCCCAGGGGC
AGCGAGGGCGCTCTCGGGATGCGGCTCCCTGTGCGGCACACACC3'

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

5' CCGTCGGGTAGCGGGACGGAGATTATTGGTTGTTAGGGATTATGTAGGGTTTGGTTCGAGTTAGGGGT
AGCGAGGGCGGTTTTCGGATGCGGTTTTTTGTGCGGTATAATTT3'

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' CCACGGACGATCCGCAACGC/3C6/ (SEQ ID NO:271)

FIG. 1 (cont'd)

SHROOM1 Target DNA: (SEQ ID NO:272)

5' CCGGAGCACTCGCCGCTGCGGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAG3'

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

5' TCGGAGTATTCGTCGTTGCGCGTTTGAAGTCGTTGGCGGTAGGCGGTTTCGAG

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' CGCCGAGGCGGTTTGAAGT/3C6/ (SEQ ID NO:276)

FIG. 1 (cont'd)

SKI Target DNA: (SEQ ID NO:277)

5' C C C G G G C C T A C G G T C C T C C C G C C A C C T C C A C G G G G C G C T G T T G G G G C C C A C C A G G C A G A G C C C G T G T T C T C A G G
C G T T G G C T C A T G G A G G T G G 3'

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

5' T T C G G G T T A C G G T T T T T C G T T A T T T T A C G G G C G G T G T T G G G G T T T A T T A G T A G A G T C G T G T T T T A G G
C G T T G G T T T T A T G G A G G T G G 3'

PCR and Flap Assay Oligonucleotides:

SKI Forward Primer: 5' A C G G T T T T T C G T T A T T T T A C G G G 3' (SEQ ID NO:279)

SKI Reverse Primer: 5' C A A C G C C T A A A A A C A C A C G A C T C 3' (SEQ ID NO:280)

SKI Flap oligo.: 5' C G C C G A G G G C G G T T G T T G G / 3 C 6 / (SEQ ID NO:281)

FIG. 1 (cont'd)

S1PR4 Target DNA: (SEQ ID NO:282)

5' GGGCCTGTCCCGTTCCCTGCTCCCATACAGGCGAGGCTGCGTGCACACAGCTTCCTGTACCCAGGAGGCCCTG
CCTGGCACGCCCGGTGGCTGCACCATCCACACGCAAGACTGCAACTTCAGATGCTCCGCACGCTGGAGATG3'

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

5' GGGTTTGTTCGTTTTTTTATATAGGCGAGTTGCGTGTATATAGTTTTTGTATTTAGAGGGTTTG
TTTGGTACGTATTCCGGTGTGTATTATTATACGTAAGATTGTAATTTTAGATGTTTCGTACGTTGGAGATG3'

PCR and Flap Assay Oligonucleotides:

S1PR4_HM Forward Primer: 5' TTATATAGGCGAGTTGCGT3' (SEQ ID NO:284)

S1PR4_HM Reverse Primer: 5' CTTACGTATAAATAATACAACCCGGAATA3' (SEQ ID NO:285)

S1PR4_HM Flap oligo.: 5' CCACGGACGACGTACCAACA/3C6/ (SEQ ID NO:286)

FIG. 1 (cont'd)

SLC12A8 Target DNA: (SEQ ID NO:287)

5' CGGAGCTAGGAGGGTGGGGCTCGGAGGGCGCAGGAAGAGCGGCTCTGCGAGGAAAGGAAAGGAGAGGCCGCTTC
TGGGAAGGGACCC3'

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

5' CGGAGTTAGGAGGGTGGGGTTCGGAGGGCGTAGGAAGAGCGGTTTTCGAGGAAAGGAAAGGAGAGGTCGTTT
TGGGAAGGGATT3'

PCR and Flap Assay Oligonucleotides:

SLC12A8 Forward Primer:	5' TTAGGAGGTGGGGTTCG3' (SEQ ID NO:289)
SLC12A8 Reverse Primer:	5' CTTTCCCTCGCAAAACCGC3' (SEQ ID NO:290)
SLC12A8 Flap oligo.:	5' CCACGGACGGAGGGCGTAGG/3C6/ (SEQ ID NO:291)

FIG. 1 (cont'd)

SOBP Target DNA: (SEQ ID NO:292)

5' GCCCCGGGGCCCCGAGGGGGCCCGGGCCCTGCAACGTCAATCGTGAACGGCACGGCGGG3'

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

5' GTTTCGGCGGTTTCGAGGCGGTCGCGGTTTGTAAAGTTATCGTGAACGGTACGGCGGG3'

PCR and Flap Assay Oligonucleotides:

SOBP_HM Forward Primer:	5' TTTCGGCGGTTTCGAG3' (SEQ ID NO:294)
SOBP_HM Reverse Primer:	5' CGTACCGTTTCACGATAACGT3' (SEQ ID NO:295)
SOBP_HM Flap oligo.:	5' CGCCGAGGGCGGTCGCGGT/3C6/ (SEQ ID NO:296)
SOBP_HM Flap oligo. v2:	5' CGCCGAGGTTACAAACCGCG/3C6/ (SEQ ID NO:297)

FIG. 1 (cont'd)

SPOCK2 Target DNA: (SEQ ID NO:298)

5'CTAGGCGAGATGGTGGAAAGCGGTGTCCGTACGGGGGTGGGCTGGGGTCCCCGTGCAGAAAGGCCGCCGAGGACCC
AGGCTGGTTTCCCC3'

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

5'TTAGGCGAGATGGTGGAAAGCGGTGTCCGTACGGGGGTGGGTGGGGTTTTCGTGTAGAAAGGCCGCCGAGGATTT
AGGTTGGTTTTTTT3'

PCR and Flap Assay Oligonucleotides:

SPOCK2 Forward Primer: 5'CGAGATGGTGGAAAGCG3' (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' GCGCTGCTGCGCCGCCAGGCAAGGCGAGGGTCCGGGAGAAAGGCTCGGCTCCCTCCTAAACATGTGGCCCCGTGGCG
TCCCCCTGTCCCCCTCCGAGCGATGCTCCTGCGCCCTTCGCCGCTGCTGCGCGCTGCTGCGCCGCCAGGCAA3'

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

5' GCGAGGGTTCGGGAGAGGTTTCGGTTTTTTTAAATATGTGGTTCGTGGCGTTTTTTTGTTCGAGCGA
TGTTTTGCGTTTTTCGTCGTTTTTCGCGTTGTTCGCTCGTAGGTAA3'

PCR and Flap Assay Oligonucleotides:

ST8SIA1 Forward Primer: 5' AAATATGTGTTTCGTGGCGTT3' (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'ACGAGAAAGAGATCGTGACAGGGGGTGCTGCAACAGGGCACGGCGTGGAGGAGGAACCCAGACCCGGGCCAGAGCGT
TCAGGTACTCCTGCCCTCGCGGCTCCTCCCCCTCTAGCGTCCTTCCCTCCCCGAGTGCAGAGG3'

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

5'ACGAGAAAGAGATCGTGAGGGGGTGTGTAATAGGTACGGCGTGGAGGAGGAATTAGATCGCGGTAGAGCGT
TTAGGTATTTTGTTCGCGGTTTTTTTTTTTAGCGTTTTTTTTTTTCGAGGTAGAGG3'

PCR and Flap Assay Oligonucleotides:

ST8SIA1_22 Forward Primer:	5'GGGGTGTGTAATAGGGTACG3' (SEQ ID NO:310)
ST8SIA1_22 Reverse Primer:	5'CTAAACGCTCTAACCGCGA3' (SEQ ID NO:311)
ST8SIA1_22 Flap oligo..	5'CCACGGACGGCGTGGAGGAG/3C6/ (SEQ ID NO:312)

FIG. 1 (cont'd)

SP9 Target DNA: (SEQ ID NO:313)

5' CGCGCCGTTGGTCACCTCGCCGGCCGACCGTCGAATGGAAGCCCGACTTGTACCAGGACTCGTACGGGTGCGC
CATGCCACGCGGGTACAGCCCGTCGGCTGCCGTCGTGTG3'

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

5' CGCGTCGTTGGTTATTCGTGGTCGTTAGCGTCGAATGGAAGTTCGATTGTATTAGGATTCGTACGGGTGCGT
TATGTTACGCGGGTATAGTTCGTGGTTGTCGTCGTGTG3'

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'GGTTCCCTCCCGTGGGTTCTTAATCGTCTCGCTGACTTCCAGAAATGAAACTGCAGACCCCTCGCGGTAAAGATGGC
GTGACCAGAA3'

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

5'GGTTTTTTTCGTGGGTTTTTAATCGTTTCGTTGATTTTAGAATGAAATTGTAGATTTTCGCGGTAAAGATGGC
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'CCACGGACGCGAAATCTACA/3C6/ (SEQ ID NO:323)

SUCLG2_HM For. Primer Univ.: 5'GGTTTTTTTCGTGGGTTTTTAATCG3' (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' GGAGTGAGTGCCCTACAACGCGCAGGCCGGACTGATCCCCCGTTGCTGCAGGTTGGTGCCCCCAAGCTGCGGGTGCT
CGGGCGCCAACTAAAGCCAGCTCTGTCCAGACGCGGAAAG3'

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

5' GGAGTGAGTGTATAACGCGTAGTCCGGATTGATTTTCGTTGTAGGTTGGTGTTTAAGTTGCGGGTGTT
CGGGCGTTAATTAAAGTTAGTTTGTGTTAGACGCGGAAAG3'

PCR and Flap Assay Oligonucleotides:

TBX15 Forward Primer:	5' CGTAGGTCGGATTGATTTTCGT3' (SEQ ID NO:329)
TBX15 Reverse Primer:	5' TCTAAACAAACTAACTTTAATTACGCCC3' (SEQ ID NO:330)
TBX15 Flap oligo.:	5' CCACGGACGCGAACAACCCGCA/3C6/ (SEQ ID NO:331)

FIG. 1 (cont'd)

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

5' GGAAGGAAATTGCGGGTCCCGTCTGCCTTGCTCCAGCTTCTCTGCTGAAGCCCGTAGCAGTGAATGCGCGCT
GACTTTCAGCGACGACTCCTGGAAGCAACGCCA3'

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

5' GGAAGGAAATTCGGGTTTTTCGTTTGTGTTTTTAGTTTTTGAAGTTCGGTAGTAGTGAATGCCCGTT
GATTTTAGCGACGATTTTGAAGTAACGTTA3'

PCR and Flap Assay Oligonucleotides:

TBX15 Reg.	2	Forward Primer:	5' AGGAAATTCGGGTTTTCG3' (SEQ ID NO:332)
TBX15 Reg.	2	Forward Primer	Univ.: 5' GGAAGGAAATTCGGGTTTC3' (SEQ ID NO:333)
TBX15 Reg.	2	Reverse Primer:	5' CCAAAAATCGTCGCTAAAATCAAC3' (SEQ ID NO:334)
TBX15 Reg.	2	Flap oligo.:	5' CCACGGACGCGGCATTCAC/3C6/ (SEQ ID NO:335)

FIG. 1 (cont'd)

TRH Target DNA: (SEQ ID NO:336)

5' GGCGCGCGACCCCTCCCGCTGACCTCACTCGAGCCGCGCCTGGCGCAGATATAAGCGCGGCCCATCTGAA
GAGGCTCGGCAGCGCGCCCGGGTC3'

Bisulfite-converted Target DNA with primer and Flap oligo. sites
(SEQ ID NO:337)

5' GGCGGTCGCGATTTTTCGTTGATTTTATTCGAGTCGTCGTTTGGCGTAGATATAAGCGCGGTTTATTGAA
GAGGTTCCGTTAGGCGTTCGGGGTT3'

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' CGCCGAGGTCGTTTGCGGT/3C6/ (SEQ ID NO:340)

FIG. 1 (cont'd)

TSC22D4 Target DNA: (SEQ ID NO:341)

5'CGGGTGGTGAAGCTGCCCCACGGCCTGGGAGAGCCTTATCGCCCGGTCGCTGGACGTGTGGATGTTTATGAG
CGAGACCTGGAGCCCCACAGCTTCGGCGGACTCCTGGAGGAA3'

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

5'CGGGTGGTGAAGTTGTTTACGGTTTGGGAGAGTTTATCGTCGCGGTCGTTGGACGTGTGGATGTTTATGAG
CGAGATTTGGAGTTTATAGTTTCGGCGGATTTTGGAGGAA3'

PCR and Flap Assay Oligonucleotides:

TSC22D4 Forward Primer: 5'GTTTGGGAGAGTTTATCGTCG3' (SEQ ID NO:343)

TSC22D4 Reverse Primer: 5'CCTCCAAAATCCGCCGA3' (SEQ ID NO:344)

TSC22D4 Flap oligo.: 5'CGCCGAGGCGGTCGTGGA/3C6/ (SEQ ID NO:345)

FIG. 1 (cont'd)

ZDHHC1-zincfinger, DHHC-typecontaining1 Target DNA: (SEQ ID NO:346)
 5' GGGGCGGGCCGACAGCCACGCTGGCGCGGCAGGCGCGTGCGCCCGCGTTTCGTGAGCCCCGAGCAG3'

Bisulfite-converted Target DNA: (SEQ ID NO:347)
 5' GGGGTCGGGTCGATAGTTACGTTGGCGCGGTAGGCGCGTGCGTTTCGTGAGTTCGAGTAG3'

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' GGAGCCCCAGCCCCACGCGGGCACACGAGGGTGGGTGGTCACGCCCCGAGGGTCCGCCGAGCGCGGCAGAGC
GCGGGCCGTGGGAAGTTTCTC3'

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

5' GGAGTTTTTAGTTTACGCGGGTATACGTAGGGTGGGTGGTTACGTTCTAGGGTTCGCCGAGCGCGGTAGAGC
GCGGGTCGTGGGAAGTTTTT3'

PCR and Flap Assay Oligonucleotides:

ZMIZ1 Forward Primer: 5' GTAGGGTGGGTGTTACG3' (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'GGCGCCGCCATTGCGGTCCTCATTTTGCTGCTGGTGGGTGGGCTACAGCAGGCCCTCTGGAGCCACACCCAGGGCA
CGGAGTGGGTGCAGGACCGTCACCGCGCCTTCACACGCACCATAGTGCCCC3'

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

5'GGCGTCGTTATTGCGGTTTTTATTTTGTGTTGGTGGGTGGGTATAGTAGGTTTTTGGAGTTATATTAGGGTA
CGGAGTGGGTGTAGGATCGTTATCGCGTTTTTATACGTATTATAGTGT3'

PCR and Flap Assay Oligonucleotides:

ZNF132 Forward Primer v2: 5'TGGAGTTATATTAGGGTACGGGA3' (SEQ ID NO:358)

ZNF132 Reverse Primer: 5'ACACTATAATACGTATAAAAACGCCGATA3' (SEQ ID NO:359)

ZNF132 Flap oligo.: 5'CCACGGACGAACGATCCCTAC/3C6/ (SEQ ID NO:360)

FIG. 1 (cont'd)

ZNF329 Target DNA: (SEQ ID NO:361)

5' GCGGCGAGGGCGCGTCCGCGGGTGGGTTTCACCTGGGTGGTGGGCATGTCGGGCCCGCTAGGGCGAGGGTCTG
GCCAGGGCGTAGTTCTCCTGGTGGTGGGACGCTCCGTGGCGATTGGGGTCACTCCTCTGAGG3'

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

5' GCGGCGAGGGCGCGTTCGCGGGTGGGTTTATTGGGTGGTGGTATGTCGGGTCGTTAGGGCGAGGGTTTG
GTTAGGGCGTAGTTTTTTGGTGGTGGGACGTTTCGTGGCGATTGGGGTATTTTTTGGAGG3'

PCR and Flap Assay Oligonucleotides:

ZNF329 Forward Primer: 5' GGTGTGGGTATGTCGG3' (SEQ ID NO:363)

ZNF329 Reverse Primer: 5' CCAATCGCCACGAAACG3' (SEQ ID NO:364)

ZNF329 Flap oligo.: 5' CCACGGACGGGTCGTTAGGG/3C6/ (SEQ ID NO:365)

FIG. 1 (cont'd)

ZNF671 Target DNA: (SEQ ID NO:366)

5' CCGTGGGCGCGGACAGCTGCCGGGAGCGGCAGCGGTCTCGATCGGGGACGCAGGCACTTCCGTCCCTGCAGAGCA
TCAGACGGTCTCGGGACACTGGGGACAACATCTCCTCCGGG3'

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

5' TCGTGGGCGCGGATAGTTGTCCGGAGCGGTAGCGTTTCGATCGGGACGTAGGTATTTTCGTTTTGTAGAGTA
TTAGACGGTTTCGGGATATTGGGATAATATTTTTCGGG3'

PCR and Flap Assay Oligonucleotides:

ZNF671 Forward Primer: 5' GTTGTCCGGAGCGGTAGG3' (SEQ ID NO:368)

ZNF671 Reverse Primer: 5' CCAATATCCCGAAACGCGTCT3' (SEQ ID NO:369)

ZNF671 Flap oligo.: 5' CCACGGACGGCGTTTCGATCG/3C6/ (SEQ ID NO:370)

FIG. 1 (cont'd)

ZNF781:Chr19:38183137-38183018 (GRCh37/hg19) Target DNA: (SEQ ID NO:371)
 5' AAGCTGCGCCCGGAGACGTGGGAGCGTTCTCTTGTTTCCGAGTGCGGGACTCATCGGGTCACAGTTTATGCTT
 TTATGACGCGGTGAGTCCAGCCACTGATTCCTAACGGTTTAGAGT3'

Bisulfite-converted Target DNA: (SEQ ID NO:372)
 5' AAGTTGCGTTCGGAGACGTGGGAGCGTTTTTTTGTTTTTCGAGTGCGCGGATTATCGGGTTATAGTTTATGTTT
 TTATGACGCGGTGAGTTAGTTATTGATTTTAAACGGTTTAGAGT3'

PCR and Flap Assay Oligonucleotides:

ZNF781 Forward Primer: 5' CGTTTTTTTTCGAGTGC3' (SEQ ID NO:373)
 ZNF781 Reverse Primer: 5' TCAATAACTAAACTCACCGCGTC3' (SEQ ID NO:374)
 ZNF781 Flap oligo.: 5' CCACGGACGGCGGATTATTCG/3C6/ (SEQ ID NO:375)

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	SA/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_01178094;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	SA/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	ITPR1L1	NM_178495;NM_001163524;NM_01008949;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	SA/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	SC/BC	UNDB/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-hydrolyzing)
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	neurtin 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, (mII) 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	SO/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	DNAJC8	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	IgLOX 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_00127612	-	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	SO/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_01178094;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	natrictic peptide receptor C/guanylate cyclase C (atriuretic 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	SO/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	interixin 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	SNIG2	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	SO/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	14926895	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/l) 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	LOC1511174	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	SO/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_01127221;NM_000068;NM_001174080	-	1	108;108;108;108;108	1	773	calcium channel, voltage-dependent, P/Q type, alpha 1A
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_03030	-	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	SO/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_01127221;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	SO/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.chr1.32237893-32237988	-	-	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	dynamitin 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_00119859	-	1	687;687	0	284	angiotensinogen 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	SO/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_01145661	-	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	SO/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	FOXO1	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	sepin 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	SO/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	SRX (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	SERPIN9	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_01178094;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_01178094;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	SO/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	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;8432;-884;1098	1	3955	LFNG O-fucosyltransferase
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_028830	+	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	SO/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_01178091	-	1	353;353;353	1	586	branched chain amino-acid transaminase 1, cytosolic
SC261	12	BCAT1	NM_001178092;NM_005504;NM_01178091	-	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-neuraminidase 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-sialyltransferase 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	SO/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	GMD5	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_0043355;NM_01025159	-	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_01178094;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	RHO	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	SO/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%	5%	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	CCN2	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.chr10.1193312919-1193312997	-	-	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

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, chr5.771140667-771140774	-	-	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	IL411	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_01174067;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	SO/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	17895756	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	29588455	29588550	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;565	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	MIN1	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;428	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_01128325	-	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

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_001190468;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-obliteror 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_001190468;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;9300	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	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, (mII) 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	SC/BC	UVD/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	GTTCGGGAACGGCTCTTGGGGCGTTCCAGCCCGGAGGAGTCCCGCGGCAATTTGCATGGGGC TCATTTGCATGACCCCGCCCGCGGGAGTCGGGGCGC
SEQ ID NO:2	AGRN Converted DNA	GTTTCGGAACGGTTTTTGGGGCGTTTAGTTTTACGGATTCTGAGGAGTTTTCTCGTAATTTGTATGGGGTTT ATTTGTATGATTTCTGCGCGGGAGTCGGGGCGT
SEQ ID NO:3	AGRN Forward Primer	GGCGTTTAGTTTACGGATTCTG
SEQ ID NO:4	AGRN Reverse Primer	ACAAATAAACCCCATACAAATTACGAC
SEQ ID NO:5	AGRN Flap oligonucleotide	CGCGAGGCGGAAACTCCT/3C6/
SEQ ID NO:6	ANGPT1 Target DNA	CGGATTCAACATGGCAATGTGCCACACTTCTCCAGAACACGATGGCACTGTCGTGAGAGTACGACAGA CCAGTACAAACACAAACGCTCTGCAGAGAGATGCTCCACACGTGGAACCG
SEQ ID NO:7	ANGPT1 Converted DNA	CGGATTTAATATGGTAATGTGTTTATATTTTATTTTATAGATACTGTTAAATTTCTGAGAGTACGATAGAT TAGTATAATATAACGTTTTGTAGAGAGATTTTTATACGTGGAATCG
SEQ ID NO:8	ANGPT1 Forward Primer	TTTTAGAACGATGGTAATTTGTCGT
SEQ ID NO:9	ANGPT1 Reverse Primer	ACATCTCTACAAAACGTTTATATATACTAATC
SEQ ID NO:10	ANGPT1 Flap oligonucleotide	CGCGAGGCTACTCT/3C6/
SEQ ID NO:11	ANKRD13B Target DNA	GGAGTACGACGACGCTGCGGCTGGCATGGAATGTCGGCGAGGAGGAGGCGGGCGCGC GGCCAGGAGGAGGAGGAGCTGGAGCGCATCTGAG
SEQ ID NO:12	ANKRD13B Converted DNA	GGAGTACGACGAGTGTGGGTTGGCGATGGAATTTGTCGGCTAGGAGTAGGAGGCGGGCGCGCGG CGTTAGGAGGAGGAGGTTGGACGTTATTTGAG
SEQ ID NO:13	ANKRD13B Forward Primer	AGTTACGACGATGTTGCG
SEQ ID NO:14	ANKRD13B Reverse Primer	TCCTCTACTCTACGCC
SEQ ID NO:15	ANKRD13B Flap oligonucleotide	CCACGGACGCAATTCAT/3C6/
SEQ ID NO:16	ARHGEF4 Target DNA	GGTGGCAACGGCTGGAGTCCGTCGCCCGGCCACTACCCCGCGCGGCCCTGCGCGGCCGCTCAGCGGAAG GCCAGCAGGAAGATCAGTACGACGTTGATAGAAACGAGGCCAGCGCGGAGACCAACCGCG
SEQ ID NO:17	ARHGEF4 Converted DNA	GGTGGTAACGGTTGGAGTGTCTGCTCGGTTATTTATTCGGCGCGGCTTTTTCGGCGGAGGT TAGTAGGAAGATTAGTACGACGTTGATGAGAAATTAGGAGGTTAGTACGGGAGATTATTACGCG
SEQ ID NO:18	ARHGEF4 Forward Primer	CGTTCGCGTTATTATTTCGGCG
SEQ ID NO:19	ARHGEF4 Reverse Primer	GCTCTAATTCATCAACGTCGT
SEQ ID NO:20	ARHGEF4 Flap oligonucleotide	CGCGAGGGCGCGTTTTC/3C6/
SEQ ID NO:21	BARX1 Target DNA	GGCCCGGGCGCTGGGCCCTAGGGCTGGACGTCAACCTGTTAGATAGAGGCGTGGGACCCCGCAGGCG GCTGCTCGACGACCGCATCCGGAG
SEQ ID NO:22	BARX1 Converted DNA	GGTTCGGGTGCTTTGGGTTTTAGGGTTTGACGTTAAATTTTGTAGATAGAGGCGTGGGATTTTTCGTAGGCGG TTGTCGACGATCGTATTCGGAG
SEQ ID NO:23	BARX1 Forward Primer	CGTTAATTTGTAGATAGAGGCG
SEQ ID NO:24	BARX1 Reverse Primer	ACGATCGCCGAACAC
SEQ ID NO:25	BARX1 Flap oligonucleotide	CCACGGACGCGCTACGAAAA/3C6/
SEQ ID NO:26	BARX1 Reverse Primer Universal	TCCGAACAACCCGCTAC

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ.ID NO:27	BARX1 Flap oligonucleotide Universal	CCACGGACGCGAAAAATCCCA/3C6/
SEQ.ID NO:28	BCAT1 Target DNA	GCTTCACGCGGCGCTCGTGCCACTGCGGCTCTCTGAGCCCGCGCTCCCGCAGCCTCCCCATGGCCAGCCCGC TTCGCTCCGCTGCGGCCCTTGCCGCCAGGTACCTCGAACCC
SEQ.ID NO:29	BCAT1 Converted DNA	GTTTTAGTCGCGCTTTCGTATTATGTCGTTTTTTGATGTTTCGCGTTTTTCGTAGTTTTTTTATGTTAGTTCGTTTT GTTTCGTTGCGGTTTTTGTCGTTAGGTATTTCGAATTT
SEQ.ID NO:30	BCAT1 Forward Primer	GTGTTATTGTCGTTTTTTGTAGTTTCG
SEQ.ID NO:31	BCAT1 Reverse Primer	CGCAACGAAACGAAACGA
SEQ.ID NO:32	BCAT1 Flap oligonucleotide	CGCGAGGGCGTTTTTCGTAG/3C6/
SEQ.ID NO:33	BCL2L11 Target DNA	GCCGCCGCACGCCGAATGCTCCGCGCTCCCGGGGTCCCGGAGCTCAGACAGGACCGGAAAGAACCAACG CAGAAGAAAGCCCTATTCTTGCTGCTGTTCTGTGACGCTTGCAGCCCTGCGCCGCCGCGT
SEQ.ID NO:34	BCL2L11 Converted DNA	GTTGCTGTCAGTCGTAATGTTTCGCGTTTTTCGCGGGTCTGGGCGATTAGATAGGGATCGGAAAAAGAAATTACGTA GAAGAAAGTTTTATTTTTTTGCTGTTGTTTTTGTAGTTTTGTCGTTTCGTTTTTCGCGT
SEQ.ID NO:35	BCL2L11 Forward Primer	CGTAATGTTTCGCGTTTTTCG
SEQ.ID NO:36	BCL2L11 Reverse Primer	ACTTCTCTACGTAATTCTTTTCGA
SEQ.ID NO:37	BCL2L11 Flap oligonucleotide	CGCGAGGGCGGGGTCGGGC/3C6/
SEQ.ID NO:38	BHLHE23 Target DNA	GCCGGGGAGTCAGAAAGCAAGTACTAGCGCTCAGGACCGCGCGCGCCGCCCGCGCGCCGCCCGCCCTC GGTCCAGAGC
SEQ.ID NO:39	BHLHE23 Converted DNA	GTGCGGGAGTCAGAAAGTAAGTATTAGCGTTTTAGGATCGCGCGCTGTTTCGCGTCTGTTTCGCGTCTTTTTTCGG TTTAGAGT
SEQ.ID NO:40	BHLHE23 Forward Primer	AGTATTAGCGTTTAGGATCGCG
SEQ.ID NO:41	BHLHE23 Reverse Primer	ACTCTAAACCGAAAAACGACG
SEQ.ID NO:42	BHLHE23 Flap oligonucleotide	CCACGGACGGCGAAACGACGC/3C6/
SEQ.ID NO:43	BIN2 Target DNA	GCCGGGAGCCCGCACTTCTCTCGGGGCTCAGAAAAACACAGGGCGGGGCCAGGGCGGGGCCCC CAGG
SEQ.ID NO:44	BIN2 Converted DNA	GTGCGGGAGTTCGTATTTTTTTTCGGGGTTTTAGAAAAATTATAGGCGCGGGGTTAGGGCGGCGGTTTT TAGG
SEQ.ID NO:45	BIN2 Forward Primer	TCGGGAGTTCGTATTTTTTTTTTCGG
SEQ.ID NO:46	BIN2 Reverse Primer	AAAAACGCGCCCTAAC
SEQ.ID NO:47	BIN2 Flap oligonucleotide	CGCCGAGGCCCGCGCCCTA/3C6/
SEQ.ID NO:48	BIN2_Z Target DNA	CGGGGCCTACCTCAGGCAGCGCTCGCTCGAGGCCAGCTTCGAGCTCAACCCCTGCCCGAAACCTCGGCCTCACT G
SEQ.ID NO:49	BIN2_Z Converted DNA	CGGGGTTATTTTAGGTAGCGTTCGTTTCAGAGTTAGTTTTTCGAGTTTTTAATTTTTGTTTCGAAATTTTCGGTTTTATTG
SEQ.ID NO:50	BIN2_Z Forward Primer	GGGTTTATTTTAGGTAGCGTTCG
SEQ.ID NO:51	BIN2_Z Reverse Primer	CGAAATTCGAACAAAAATTAAACTCGA
SEQ.ID NO:52	BIN2_Z Flap oligonucleotide	CCACGGACGTTTCAGGTAG/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:53	CAPN2 Target DNA	TGTCCTGACACGATGGCCACAGGACAGTTTGTGTGATGCCAGGGGCGCGGCCACCGGTGGTCCAGTTT ACACTCGGCCCGCACTCTGAAGTCCGCGGAGGAGAAAGGCGTCCCTTCGCAGCTCGG
SEQ ID NO:54	CAPN2 Converted DNA	TGTTTTATACGATGGTTATAGGTATAGTTGTGTGATGTTTAGGGGTCGCGCGGTTTACGGTGGTTAGTTTAT ATTCGGGTTTCGTAATTTGAAGTTTCGCGGGAGGAGAGGGCGTTTTTTTCGTAGTTCGG
SEQ ID NO:55	CAPN2 Forward Primer	TGATGTTAGGGGTTTCGCG
SEQ ID NO:56	CAPN2 Reverse Primer	CGAAACTCAAAAATACGAAACCCGA
SEQ ID NO:57	CAPN2 Flap oligonucleotide	CGCCGAGG GCGGTTTACGG/3C6/
SEQ ID NO:58	chr5_132 Target DNA	CCGGAGCACTGCGCGCTGCGCGCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAAGCCGCGGCTGGGCGGCTC GGCAGCCTGCGCGCGCGCTCGCGCTCGCGCCGCGCAGC
SEQ ID NO:59	chr5_132 Converted DNA	TCGGAGTATTCGTGCTGCGGCTTTGAAGTCGTTGGCGGTAGGCGGTTTTCGAGGTCGGCGGTTGGGCGGTTCCG GTAGTTTGGTGGCGGTTTTCGTTTCGGTCTGTAGT
SEQ ID NO:60	chr5_132 Forward Primer	GTATTCGTGTTGCGCG
SEQ ID NO:61	chr5_132 Reverse Primer	CCTCGAAACCGCCTACC
SEQ ID NO:62	chr5_132 Flap oligonucleotide	CCACGGACGCGCCACGACTT/3C6/
SEQ ID NO:63	chr7_636 Target DNA	CGCGTGAGTGTTATAGTCTCTAAAGCGCGGTGTCGGAGTTTCTTCTGTGGGTTCGTGTCGCCGCGC TCAGGAGTGAAGTCAGATCTTCGCGGTGAGTGTACAGCTCCTAAGGCGGCGCAT
SEQ ID NO:64	chr7_636 Converted DNA	CGTCGTAGTGTTATAGTTTTAAAGCGCGGTGTCGGAGTTTTTTTTTTTGGTGGGTTCTGTGTTTCGTGCGTT TAGGAGTGAAGTTGATGTTTTTCGCGGTGAGTGTATAGTTTTTAAAGCGCGCGTAT
SEQ ID NO:65	chr7_636 Forward Primer	TAAAGCGCGGTGTTTCG
SEQ ID NO:66	chr7_636 Reverse Primer	CAACTCACTCTAAACCGAC
SEQ ID NO:67	chr7_636 Flap oligonucleotide	CCACGGACGGAACACGAA/3C6/
SEQ ID NO:68	CYP26C1 Target DNA	AACGCGCTTCTGGCTACTCGGAATCGCCAGCAGATGAGGCCAGACCGCGCCGCGCTGATCAGCGCGCTCC CACAGGTCCTGGCGCGGTGTTAGCGCGCGC
SEQ ID NO:69	CYP26C1 Converted DNA	AATTGTTTTTTGGTATTTCGGAATCGTTAAGTAGATGAGGTAGATCGTCTGATTACGCGCGTTTTTA TAGGTTTTGGCGCGGTGTTTAGTCGCGT
SEQ ID NO:70	CYP26C1 Forward Primer	TGGTTTTTGGTTATTTCGGAATCGT
SEQ ID NO:71	CYP26C1 Reverse Primer	GCGCGTAATCAACGCTAAC
SEQ ID NO:72	CYP26C1 Flap oligonucleotide	CGCGGAGGCGAGCATCTAAC/3C6/
SEQ ID NO:73	DIDO1 Target DNA	GGAGCGGCGAGAGGAGGAGCCGCGCGAGGCCAGGCGCGCCCTCGCCCTCGCCCTCGCCCTCCCTCC GCTGCTCCCC
SEQ ID NO:74	DIDO1 Converted DNA	GGAGCGGCTAGAGGAGGAGTTTAGCGTCGAGGTTTAGGCGGTTTCGTTTTTCGTTTTTTTCGTT GTTTTTT
SEQ ID NO:75	DIDO1 Forward Primer	GAGGAGGATTTAGCGTCG
SEQ ID NO:76	DIDO1 Reverse Primer	CACGAAAAAAGCGAAACGAAAC
SEQ ID NO:77	DIDO1 Flap oligonucleotide	CGCGGAGGCGCCTAAC/3C6/
SEQ ID NO:78	DLX4 Target DNA	GCGGTCTATACGGGCAACCCCTAACACTTGGTAGTGCAGTGTCTCTGGCAGTCTCTGGGCTCCATACGATGCCT ACGCACGCCCTAGCAGAGGAGGTCTCTGT

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:79	DLX4 Converted DNA	GCGTTTATTACGGGTATTTTAAATATTTGGTGAGTGCAGTGTTCGAGTGTTCGGGTTTATACGATGTTTAT
SEQ ID NO:80	DLX4 Forward Primer	CGTACGTTTTAGTAGAGGAGGTTTTTGT
SEQ ID NO:81	DLX4 Reverse Primer	TGAGTGCAGTGTTCG
SEQ ID NO:82	DLX4 Flap oligonucleotide	CTCCTCTACTAAACGTACGATAAACA
SEQ ID NO:83	DLX4 Forward Primer Universal	CGCCGAGGATCGTATAAAAC/3C6/
SEQ ID NO:84	DLX4 Reverse Primer Universal	ATATTTGGTGAGTGCAGTAGT
SEQ ID NO:85	DLX4 Flap oligonucleotide Universal	ACGTACGATAAAACATCGTATAAAACC
SEQ ID NO:86	DMRTA2 Target DNA	CGCCGAGGTTTTTCGGTAGT/3C6/ TACTCCACTGCCGGTTGGTCCCAAGCTCGGCTCCGCCACCATGGACTACGCCCTTAGCGATCTCATGCGTGAC CGCTCGGCCCGCTGCTCGCGGTGCACAAAGGAGCCGACCT
SEQ ID NO:87	DMRTA2 Converted DNA	TATTTTATTGTCGGTTGGTGTTCAGTTTCGTTTTCGTTTATTTATGATTACGTTTTTAGCGATTTTATGCGTGATCG TTCGGTCTGTTGTTGCGCGGTGTATAAGGAGTGCATTT
SEQ ID NO:88	DMRTA2 Forward Primer	TGGTGTTCAGTTTCGGTTTCGT
SEQ ID NO:89	DMRTA2 Reverse Primer	CCGCAACAGCAGCACC
SEQ ID NO:90	DMRTA2 Flap oligonucleotide	CGCCGAGGGAGACATCAG/3C6/
SEQ ID NO:91	DNMT3A Target DNA	AGCGCGGTACGAACAAGCGTGGCAGTGCGGCCGCCACCGGCACAGGTGCCGCGACAGACGCCCGCT CCCCGCCACGGCCCCCGGGCTGAGCC
SEQ ID NO:92	DNMT3A Converted DNA	AGTTCGGTTACGAATAAAGCGTTGGCAGTGCGGTTCTTTACGCGTATAGGTGTTCGCGATAAGACGTTTCGTTT TCGTTTACGGGTTTCGCGGTTGAGTT
SEQ ID NO:93	DNMT3A Forward Primer	GTTACGAATAAAGCGTTGGCG
SEQ ID NO:94	DNMT3A Reverse Primer	AACGAACGTTCTATCGCA
SEQ ID NO:95	DNMT3A Flap oligonucleotide	CCACGGACGGAGTGGCGTTC/3C6/
SEQ ID NO:96	DOCK2 Target DNA	GCCGGCCCCGACGATCCTCTGCTCGCGGCTCTCCCGCACCTGTCCCGCTCCCTGCGGCCCTGGGGCCCCGCAC CTACCCAC
SEQ ID NO:97	DOCK2 Converted DNA	GTCCGTTTCGTAGTATTTTGTTCGGGTTTTTCGTTATTTTCGTTTTCGCGTTTTTGGGGTTCGTATTTAT TTAT
SEQ ID NO:98	DOCK2 Forward Primer	CGTTTCGTAGTATTTTTCGTTTCG
SEQ ID NO:99	DOCK2 Reverse Primer	GAACCCCAAAACGCGAC
SEQ ID NO:100	DOCK2 Flap oligonucleotide	CGCCGAGGGCGTTTTTCG/3C6/
SEQ ID NO:101	DTX1 Target DNA	CGCTCTGGGTCTCCCCCGAGTGGAGGAGCGCGGTCCCGCTCCGCCCGCTCCCTCCAGGCCCTCGG CCGCCGCCGAGCTTCCGCGGTGGACAGCTCCCGGCCGACGACGAGG
SEQ ID NO:102	DTX1 Converted DNA	CGTTTTTGGGTTTTTTCGAGTGGAGGAGTGGCGGTTTCGTTTTCGCGTTCTTTTATAGGTTTTTCGGTTCG TCGCGTGAGTTTTTCGCGGTGGATAGATTGTTTCGTCGACGAGGACGAGTAGG
SEQ ID NO:103	DTX1 Forward Primer	GAGTCGGGTTTCGTTTC
SEQ ID NO:104	DTX1 Reverse Primer	GACGCGACGACGAAAAAC
SEQ ID NO:105	DTX1 Flap oligonucleotide	CGCCGAGGGCGTTTCGTTT/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:106	EMX1 Target DNA	TCCGGCGCCGCTTTCTAGAGAACCGGGTCTCAGCGATGCTCATTTTCAGCCCGCTTAATGCAACAAACGAAACC
SEQ ID NO:107	EMX1 Converted DNA	CCACACGAACGAAAGGAAACATGCTCGGCT TCGGCGTCGGTTTTTTAGAGAAATCGGGTTTAGCGATGTTTTATTTAGTTTCGTTTTAATGTAATAACGAAATTTTA TACGAACGAAAGGAATATGTTTCGTT
SEQ ID NO:108	EMX1 Forward Primer	GGCGTCGCTTTTITAGAGAA
SEQ ID NO:109	EMX1 Reverse Primer	TTCTTTTCGTCGTATAAAATTTTCGTT
SEQ ID NO:110	EMX1 Flap oligonucleotide	CCACGGACGATCGGGTTTTAG/3C6/
SEQ ID NO:111	FAM59B Target DNA	GGGCTGCTGGCCGGGACCCGCGCTCGAGCGCTGGTGCAGCGCTCTACTGCGCGAGCGCTTCGA CCCGACGAGTACTCCACGGCGTGCAGGCGCGAGCGCCAGCGAGCTCGCGAAG
SEQ ID NO:112	FAM59B Converted DNA	GGGTTGTTGGTCGGGATTTCGCGCTCGAGCGTTTTGGTGCAGATAGCGTTTTTTATTGTGCGGAGCGTTTCGATT TCGACGAGTATTTACGGTCTGTCGCGAGGCGTTAGCGGAGTTTCGTCGAAG
SEQ ID NO:113	FAM59B Forward Primer	CGATAGCGTTTTTATTGTCGCG
SEQ ID NO:114	FAM59B Reverse Primer	GCACGACCGTAAATACTCGTC
SEQ ID NO:115	FAM59B Flap oligonucleotide	CCACGGACGCGAAATCGAAAC/3C6/
SEQ ID NO:116	FERMT3 Target DNA	TAGCAGCAGCGCAGCCATGGCGGGATGAAGACAGCCTCGGGGACTACATCGACTCGTATGGGAGCTCGGG TGTTGTGGGAGAGGAGGACCCAGAGCGCGAGTCGGTCACTCGGGTCACTGGGAGTCGCAC
SEQ ID NO:117	FERMT3 Converted DNA	TAGTAGTAGTCGTAGTTATGGCGGGATGAAGATAGTTTTCGGGATTATATCGATTGTTATGGGAGTTGCGGGT GTTGTGGGAGAGGAGGATTTAGAGTTCGAGTCGTTATTTTCGGGTTATTTGGGAGTCGTAT
SEQ ID NO:118	FERMT3 Forward Primer	GTTTTCGGGATTATATCGATTTCG
SEQ ID NO:119	FERMT3 Reverse Primer	CCCAATAACCCGCAAAATAACC
SEQ ID NO:120	FERMT3 Flap oligonucleotide	CGCGGAGCGACTCGACCTC/3C6/
SEQ ID NO:121	FGF14 Target DNA	GTCCAGAGACGCCCTAGGGTCAGAGGTCTCCTCGTGGCAACGGAACCTTCCCGCTACGGCGCTCCAACGGG CCGCTCCGCGCATTCGTTAGCGAAGC
SEQ ID NO:122	FGF14 Converted DNA	GTTTTAGAGACGTTTTAGGGTTAGAGGTTATTTTCGTGTAACGGAAATTTTCGCGTTACGGCGGTTTTAACGGGT CGTTTTCGTCGTATTGCTAGCGAAGT
SEQ ID NO:123	FGF14 Forward Primer	TTTCGTGTAACGGAAATTTTCG
SEQ ID NO:124	FGF14 Reverse Primer	CGACGAAACGACCCGT
SEQ ID NO:125	FGF14 Flap oligonucleotide	CGCGAGGGCTTACGGCG/3C6/
SEQ ID NO:126	FLJ34208 Target DNA	GGCCCCGGCGCAGGAGGACAGGAGGAGGCGCACACGAGAAAGCTCCACGCGCCCGCTCGCCTCCG ACGGGAAGGCGCTCTCCGACCTCTGGATG
SEQ ID NO:127	FLJ34208 Converted DNA	GGCTTCGTGCTAGAGGAGGATAGGGAGGCGTATACGAGAAAGTTTTACGGTTCGGGTTTCGTTTCGAC GGGAAGCGTTTTTTCGATCGTTTTGGATG
SEQ ID NO:128	FLJ34208 Forward Primer	GAGCGTATACGAGAAAGTTTTACG
SEQ ID NO:129	FLJ34208 Reverse Primer	AACGCTTCCCGTCGAA
SEQ ID NO:130	FLJ34208 Flap oligonucleotide	CCACGGACG GCGTTCGCGTT/3C6/
SEQ ID NO:131	FLJ45983 Target DNA	CGAGAGGGCGGACACAGCCGAGCCATGAGGTGACGGCGGACCAGCCGCGTGGGTGAGCCACCACCC CGCGTGCTCAACGGGCGACGCCCGGACCGCAC

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:132	FLJ45983 Converted DNA	CGAGAGGGCGGAGTAGTATGTCGAGGTTATGGAGGTGACGGGGGATTAGTCGCGTTGGGTGAGTTATTATTTCG
SEQ ID NO:133	FLJ45983 Forward Primer	TCGTGTTTAACGGGTAGTATTCGGATACGTAT
SEQ ID NO:134	FLJ45983 Reverse Primer	GGGCGGAGTATAGTCG
SEQ ID NO:135	FLJ45983 Flap oligonucleotide	CAACGCGACTAATCCGC
SEQ ID NO:136	GRIN2D Target DNA	CGCGAGGGCGTCACCTCCA/3C6/ CGCCCCCTCACCTCCCGATCATCGCGTTCAGAGCGCATCGATCTTCCGTCCTTGCCATTGGTGACCAAGGTAG AGGTCGTAGCTGAAGCCGATGGTATGCGCCAGCCGCTTCAGAAATGTCGATGCAGAAACCCCTG
SEQ ID NO:137	GRIN2D Converted DNA	CGTTTTTTTATTTTCGATTATGTCGTTTTAGACGTTATCGATTTTTTTTCGTGTTTGTATTGGGTAGGTAGAG GTCGTAGTTGAAGTCGATGCGTTAGTCGTTTTAGAAATGTCGATGTAGAAATTTTTG
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/ CGCGAGGCTTCCCGCTGGCGCATACAAAGCGCTCGACCATCACCTCCAGGGAGATCCAGACGGCCGTGCGC
SEQ ID NO:141	HIST1H2BE Target DNA	CTGCTGCTCCCGGGGA
SEQ ID NO:142	HIST1H2BE Converted DNA	CGCGAGGTTTTTCGTTGGCGTATTATAAGCGTTTCGATTATTTTTAGGGAGATTTAGACGGTCTGTCGTTTT GTTGTTTTTCGGGA
SEQ ID NO:143	HIST1H2BE Forward Primer	TGGCGTATTATAAAGCGTTTCG
SEQ ID NO:144	HIST1H2BE Reverse Primer	AACAACAAACGCACGACC
SEQ ID NO:145	HIST1H2BE Flap oligonucleotide	CCACGGACGGTCTAAATCTC/3C6/ GGGCGGCCAGCGCTGGCAGCGTGATGCCACCACCTGGGGCCCTGGGCACTACTAGTGGACTCGTTCCTGC
SEQ ID NO:146	HOXA9 Target DNA	TGGCGCCGACCGCGGATGAGCTG
SEQ ID NO:147	HOXA9 Converted DNA	GGCGGGTTAGCGTTGGGTACGGTGATGTTATTATTTGGGGTTTTGGGTAATTATTACGTGGATTCTGTTTTTGTG GGCGTCGACGTCGCGGATGAGTTG
SEQ ID NO:148	HOXA9 Forward Primer	TTGGGTAATTATTACGTGGATTCTG
SEQ ID NO:149	HOXA9 Reverse Primer	ACTCATCCGCGACGTC
SEQ ID NO:150	HOXA9 Flap oligonucleotide	CCACGGACGCGACGCCCAACA/3C6/ GGGCCATTGCCAGAAGACGCTCTCTCGGGGCGCCAGGATTCACCTTTCCTCCGACCTCAACTTCTTCGGGCCGA
SEQ ID NO:151	HOXB2 Target DNA	CTCCTGTCTCCAGCTATC
SEQ ID NO:152	HOXB2 Converted DNA	GGGTATTGTAGAAGACGTTTTTTCGGGCGTTAGGATTATTTTTTTTCGATTTTAAATTTTTTCGCGGTCTGATTT TTGTTTTAGTTATT
SEQ ID NO:153	HOXB2 Forward Primer	GTTAGAAGACGTTTTTTCGGG
SEQ ID NO:154	HOXB2 Reverse Primer	AAAACAAAATCGACCGCA
SEQ ID NO:155	HOXB2 Flap oligonucleotide	CGCGAGGGCGTTAGGATT/3C6/ GGCCCCGAAGCCAGCTCCCGGCCCTGGAGCCCGCCAGCGGCGGCGGCTGCGGCGGCTGGACCTGGGCA
SEQ ID NO:156	KLHDC7B Target DNA	GTTGCTGGACGTGCTGGCCTTGCCAGCA
SEQ ID NO:157	KLHDC7B Converted DNA	GGTTTCGGAAGTTAGTTTCGGGTTTTGGAGTTCGTTACGGCGGTAGTTTTTCGGGCGGCGGTGGATTTGGGTGGTAGT TGTTGGACGTGTTGTTTTGTTAGTA

FIG. 6 (cont'd)

[illegible]

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:183	MAX_chr12.52 Forward Primer	TCGTCGTTTTTGTGTTATCG
SEQ ID NO:184	MAX_chr12.52 Reverse Primer	AACCGAAATACAACATAAAACGC
SEQ ID NO:185	MAX_chr12.52 Flap oligonucleotide	CCACGGACGCAACCCCGCAA/3C6/
SEQ ID NO:186	MAX_chr16.50 Target DNA	GGAAGGCTGCAGCGAGAGATTACATATTATCCGAGCTTAAGGAAGCCCGCGATAATGCAGGTACAGCCCGAAACC CACGCCCCAGACCTTATCTGCGGCCCGCC
SEQ ID NO:187	MAX_chr16.50 Converted DNA	GGAAGGTTGTAGCGAGAGATTATATATTTATTCGAGTTTAAGGAAGTCGCGATAATGTAGGTATAGTTTCGAAATTT ACGTTTTAGATTTTATTGCGCGTTTCGTT
SEQ ID NO:188	MAX_chr16.50 Forward Primer	TTCGAGTTTAAGGAAGTCG
SEQ ID NO:189	MAX_chr16.50 Reverse Primer	TCTAAAAACGTAAATTTTCGAACT
SEQ ID NO:190	MAX_chr16.50 Flap Oligonucleotide	CCACGGACGCGGATAATGTAG/3C6/
SEQ ID NO:191	MAX_chr19.16 Target DNA	GGAGTTATTTTAACCATCGCTCCAGAACATACGAGCTTCTCTCTCCAACGACGAGAAACCTACTTTGGCTG TGCTTCTGTAAACACGAGGCGCTGCGATTGCTGAGAACACAGCCCGAGACTGCGCG
SEQ ID NO:192	MAX_chr19.16 Converted DNA	GGAGTTATTTTAATATCGTTTTTAGAATATTACGGAGTTTTTTTTTTTAAATACGTAGGAAATTTTATTGTTGTG TTTTTTGTTAATACGAGGTTTTGCGATTGTTGAGAAATAATAGTTTCGAGATTGCGCG
SEQ ID NO:193	MAX_chr19.16 Forward Primer	TTAATTATCGTTTTTAGAATATTACGGA
SEQ ID NO:194	MAX_chr19.16 Reverse Primer	ACTATTATCTCAACAATCGCAAAAC
SEQ ID NO:195	MAX_chr19.16 Flap oligonucleotide	CCACGGACGCTCGTATTAAAC/3C6/
SEQ ID NO:196	MAX_chr19.37 Target DNA	GGCGGGCGTTTGCCAAACCAACGAGACTCGGAATCACACTGCCACTGTGTACCTGGACGCCCATCTGCAGACC CAGCGCTGCGGGGATTCGGAAACGGGAGAGCGGGCTCC
SEQ ID NO:197	MAX_chr19.37 Converted DNA	GGCGGGCGTTTGTTAAATAGTTTAAGATTGCGGAATATATCTGTTATTGTATTGGACGTTATTGTAGATTTA GCGTTTGCGGGATTCGGAAACGGGAGAGCGGGTTTTT
SEQ ID NO:198	MAX_chr19.37 Forward Primer	AGTTTAAGATTGCGGAATTATATTCGT
SEQ ID NO:199	MAX_chr19.37 Reverse Primer	TTCCGAAATCCCGCAA
SEQ ID NO:200	MAX_chr19.37 Flap oligonucleotide	CGCCGAGGAACGCTAAATCT/3C6/
SEQ ID NO:201	MAX_chr8.124 Target DNA	CGCAGGCTGAGGCCCTCGGGTCCCGAGGGTCTCGCCATCAGTCACCTCTACGGGCCAGGCTGGGGTCACG GCCTGCAGGAGCTCCTCGCGGCCCACTCCCTCATCTCGACCCCGTGGGAGGCGACCCCTGACCCCTCGTT CCG
SEQ ID NO:202	MAX_chr8.124 Converted DNA	CGTAGGTTGAGGTTTTCGGGTTTTAGCGGGTTTTCGTTATTAGTTATTTTTACGGTTAGGTTTGGGGGTTACGGT TTGTAGGAGTTTTTTCGCGGTTTTATTTTTTATTTCGATTTCGTGGGAGGCGATTGTGATTATTTTCGTTTCG
SEQ ID NO:203	MAX_chr8.124 Forward Primer	GGTTGAGGTTTTCGGGTTTTTAG
SEQ ID NO:204	MAX_chr8.124 Reverse Primer	CTCCACGAAATCGC
SEQ ID NO:205	MAX_chr8.124 Flap oligonucleotide	CGCCGAGGGGGTTTTTCGT/3C6/
SEQ ID NO:206	MAX_chr8.124 Forward Primer v2	AGGAGTTTTTTCGCGCG
SEQ ID NO:207	MAX_chr8.124 Reverse Primer v2	ACGAAAATAATCAAAATCGCTCC
SEQ ID NO:208	MAX_chr8.124 Flap oligonucleotide v2	CGCCGAGGCCACGAAATCG/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:209	MAX_Chr8.145 Target DNA	CGGGGAGGGGGGATCAGCCAGAGCCTCAGCCGACGGCGCTCCCGAGGTCCACTTCCCGCTCCGATACCCCTCCCC
SEQ ID NO:210	MAX_Chr8.145 Converted DNA	CTAAGCACGATACCAGGGGCCAGGGCTGCTTGGCG CGGGGAGGGGGTATTAGTTAGAGTTTATGTCGACGGCGTTTTTATAGGTTTATTTTCGTTTCGATATTTTTTTTA AGTACGATATTTAGGGTTTAGGGTTGTTTTTGCGG
SEQ ID NO:211	MAX_Chr8.145 Forward Primer	GCGTATTAGTTAGAGTTTATGTCG
SEQ ID NO:212	MAX_Chr8.145 Reverse Primer	ACAACCTAAACCCCTAAATATCGT
SEQ ID NO:213	MAX_Chr8.145 Flap oligonucleotide	CCACGGACGGACGGCGTTTTT/3C6/
SEQ ID NO:214	MAX_Chr1.110 Target DNA	CTCCGCTCCCGCAGGCGCTGGCGCGCAGCGGACCAGCGGGTGTATCAATTATTCAGGCCCAAGTTCACG GGCACTGCATCCATTTCCTCGCGTGCGCC
SEQ ID NO:215	MAX_Chr1.110 Converted DNA	TTTCGTTTTTCGTAGTTTGGTCGCGCAGCGGTATTAGCGGGTGTATTAATTATTTAGGTTTTAAGTTTACGGG TATTGTTATTTATTTTTCGCGTGCCTT
SEQ ID NO:216	MAX_Chr1.110 Forward Primer	TTTCGTAGTTTGGTCGCG
SEQ ID NO:217	MAX_Chr1.110 Reverse Primer	AACCTAAATAATTAAACAACCCGC
SEQ ID NO:218	MAX_Chr1.110 Flap oligonucleotide	CCACGGACGGCAGCGGTATT/3C6/
SEQ ID NO:219	NFIX Target DNA	GTGGCGGGGGTGACGGCGGTCAAAGTCAATGATTTTCAGTTCGGTTGGCTAAACAGGGTCAGAGCTGAGA GCGAAGCAGAAAG
SEQ ID NO:220	NFIX Converted DNA	GTGGTCGGGGTGACGGCGGTAAAGTGAATGATTTTTTAGTTCGGTTGGTTAAATAGGGTTAGAGTTGAGAG CGAAGTAGAAGG
SEQ ID NO:221	NFIX Forward Primer	TGGTCGGGGCTGACGCG
SEQ ID NO:222	NFIX Reverse Primer	TCTAACCTATTAAACCAACCGA
SEQ ID NO:223	NFIX Flap oligonucleotide	CGCGAGGGCGGTAAAGTG/3C6/
SEQ ID NO:224	NKX2-6 Target DNA	GGACCTCTCGGCCCGCCCATCCGCTTCGGGATGCTGCTGAGCCCCGTACCTCCACCCCTTCTCGGTCAAGG ACATCTGCGACTGAG
SEQ ID NO:225	NKX2-6 Converted DNA	GGATTTTTCGGTTTCGTTTTATTCTGTTTTTCGGGATGTTGTGAGTTTCGTTATTTTATTTTTCGGTTAAGGATAT TTGCGATTGGAG
SEQ ID NO:226	NKX2-6 Forward Primer	GATTTTTCGGTTTCGTTTTTATTCG
SEQ ID NO:227	NKX2-6 Reverse Primer	CAATCGCAAAATATCCTTAACCGA
SEQ ID NO:228	NKX2-6 Flap oligonucleotide	CCACGGACGGTTTCGGGATG/3C6/
SEQ ID NO:229	OPLAH Target DNA	CTGTAGTGTGACCGAGCGCGCCTTCCGGCCATACGGGCTCCACGGTGC GCGGTCCCGACGCCCTCGCGGCC CTCCCCGCCGCC
SEQ ID NO:230	OPLAH Converted DNA	TTGTTAGTTGTGATCGAGCGTTCGCGTTTTTCGGTTATACGGGTTTTACGGTGC GCGGTTTTTAGTTTTTCGCGGTTTTT TTCGTTTTCG
SEQ ID NO:231	OPLAH Forward Primer	CGTCGCTTTTCGGTTATACG
SEQ ID NO:232	OPLAH Reverse Primer	CGCGAAACTAAAAACCGCG
SEQ ID NO:233	OPLAH Flap oligonucleotide	CCACGGACGGCACCCTAAAAC/3C6/
SEQ ID NO:234	PARP15 Target DNA	CGGAGTATGTTGAGGAGCGCGGGGACGGGTGCGGGAAGGGGACAGAGGGGCTGAGCCTGGGGCCCCGCAAGAC CCAGCAGCCCGAGCGGGCGCAGAGACCCACCGCCACGCACA

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:235	PARP15 Converted DNA	CGAGATATGGTGAGAGCGCGGGGACGGGTGCGGGAAGGGGATAGTAGGGTTGAGTTTGGGGTTCGTAAGATT TAGTAGTTCGAGCGGGCGTAGAGATTTTACGTTACGTATA
SEQ ID NO:236	PARP15 Forward Primer	GGTTGAGTTTGGGGTTTCG
SEQ ID NO:237	PARP15 Reverse Primer	CGTAACGTAAATCTCTACGCC
SEQ ID NO:238	PARP15 Flap oligonucleotide	CCACGGACGCGCTCGAACTAC/3C6/
SEQ ID NO:239	PRDM14 Target DNA	GGAGAGCAGCCCGCAGAACCTGGCGCGTACTACAGCCCTTCCCGTCCTATGGACACTACAGAAAACAGCCTGGCC ACCGTGAGGAAAGACTTCC
SEQ ID NO:240	PRDM14 Converted DNA	GGAGAGTAGTTCGTAGAAATTTGGTGCAGTATTATACGTTTTTTTCGTTTTATGGATATTATAGAAATAGTTTGGTTATC GTGGAGGAAGATTTTT
SEQ ID NO:241	PRDM14 Forward Primer	GAGTAGTTCGTAGAAATTTGGTTCG
SEQ ID NO:242	PRDM14 Reverse Primer	CCACGATAACCAAACTATTTCTATAATATCC
SEQ ID NO:243	PRDM14 Flap oligonucleotide	CCACGGACGCGTATTATACG/3C6/
SEQ ID NO:244	PRDM14 Forward Primer v3	GGAGAGTAGTTCGTAGAAATTTGG
SEQ ID NO:245	PRDM14 Reverse Primer v3	CTATTCTATAATATCCATAAAACGAAAAAAGCT
SEQ ID NO:246	PRDM14 Flap oligonucleotide v3	CCACGGACGTCGCGTATTAT/3C6/
SEQ ID NO:247	PRKCB_28 Target DNA	GGGAAGGTGCCCTGCGCGCGGCTCACCAGATGAAGTCGGTGCAGTGGCTGCAGAAAGTGGGCTGCTTGGAAGA AGCGGGCGGTGAATTTG
SEQ ID NO:248	PRKCB_28 Converted DNA	GGGAAGGTGTTTTCGCGCGCGGCTTATTAGATGAAGTCGGTGTAGTGGTTGTAGAAAGTGGGTTGTTTGAAGAA GCGGGCGGTGAATTTG
SEQ ID NO:249	PRKCB_28 Forward Primer	GGAAAGTGTTCGCGCG
SEQ ID NO:250	PRKCB_28 Reverse Primer	CTTCTACAACCACTACACCGA
SEQ ID NO:251	PRKCB_28 Flap oligonucleotide	CCACGGACGCGCGCGGTTTAT/3C6/
SEQ ID NO:252	PTGDR Target DNA	GCCTCGGGGCGCGGGGACTCACAAATTACGGGCAGAGAACACATAGTGAAGAGCACCGTTCATCAGCGCCAGCAGCA GGAGGTGATCCAGCTCCTCCAGGGGCTGAGGG
SEQ ID NO:253	PTGDR Converted DNA	GTTTCGGGGTTCGGGGATTATTAATTACGGGTAGAGAAATATATAGTGAAGAGTACGGTTATTAGCGTTAGTAGTAG GAGGTGATTAGTTTTTTTAGGGTTGAGGG
SEQ ID NO:254	PTGDR Forward Primer	GGGTTCGGGGATTATTAATACGG
SEQ ID NO:255	PTGDR Reverse Primer	CCTCTACTACTAACGCTAATAACC
SEQ ID NO:256	PTGDR Flap oligonucleotide	CCACGGACGCTACTCTTTCAC/3C6/
SEQ ID NO:257	PTGDR_9 Target DNA	GGCGGCTGACGGGCACCGCGCTCTCTGCACAGGGACTGTCCGAGCGCGCGGACGGGAGGAAAGCGTCC CCTCAG
SEQ ID NO:258	PTGDR_9 Converted DNA	GGCGGTTGAGCGGATTTCGCGTTTTTTGATTAGGGATTGTGTCGAGTCGCGCGGACGGGAGGAAAGCGGTTTTTT TTAG
SEQ ID NO:259	PTGDR_9 Forward Primer	GTGTAGCGGATTTCGGG
SEQ ID NO:260	PTGDR_9 Reverse Primer	CTTCTCTCCGTCGCGCG
SEQ ID NO:261	PTGDR_9 Flap oligonucleotide	CGCCGAGGCGCGACTCGACA/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO: 262	RASSF1 (Human) Target DNA	TCAGAAACACGGGTATCTCCGCGTGGTGCTTTGCGGTGCGCCGTGTCGGGTGCGGGTGGGGTGTGAGGA GGGACGAAGGAGGGAAGGAAGGCAAGCGGGGGGGCTCTGCGAGAGCGGCCAGCCCCGCCTTC
SEQ ID NO: 263	RASSF1 (Human) Converted DNA	TTTAAATACGGGTATTTTCGCGTGGTGTTTTCGCGTCGTCGTCGTTGTGTCGTTTCGGGTGGGGTGTGAGGAG GGACGAAGGAGGGAAGGAAGGTAAAGCGGGGGGGTTTTTCGAGAGCGGCTTTAGTTTCGTTTTT
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	CGGTGGGACGCGGGACGGAGATTACCTGGCTGTCCAGGGACCTTATGCAGGGTTTTGGCCCCAGCCAGGGGGC AGCAGGGGGCGTCTGCGGATGCGGCTCCCTGTGCGGCACAACACC
SEQ ID NO: 268	SHOX2 converted DNA	CGGTGGGTAGGCGGGACGGAGATTATTGTTGTTTAGGGGATTTTATGTAGGGTTTGTTCGAGTTTAGGGGTA GCGAGGGGCGTTTTCGGATGCGGTTTTTTGTGCGGTATAATATT
SEQ ID NO: 269	SHOX2 Forward Primer	GTTGAGTTTAGGGGTAGCG
SEQ ID NO: 270	SHOX2 Reverse Primer	CCGCACAAAAACCGCA
SEQ ID NO: 271	SHOX2 Flap oligonucleotide	CCACGGACGATCCGCAACGC/3C6/
SEQ ID NO: 272	SHROOM1 Target DNA	CCGGAGACTCGCCGTGCGCGCCCTGAAGCCGCTGGCGGTAGGCGGCCCTCGAG
SEQ ID NO: 273	SHROOM1 Converted DNA	TCGGAGTATTCGTCGTGCGCGTTTTGAAGTCGTTGCGGTAGGCGGTTTTTCGAG
SEQ ID NO: 274	SHROOM1 Forward Primer	GGAGTATTCGTCGTTGCG
SEQ ID NO: 275	SHROOM1 Reverse Primer	CGAAAAACCGCTACCGC
SEQ ID NO: 276	SHROOM1 Flap oligonucleotide	CGCCGAGGGCGTTTTGAAGT/3C6/
SEQ ID NO: 277	SKI Target DNA	CCCGGGCTACGTCCTCCCGCCACCTCCACGGGGCGGCTGTGGGGCCCCACCAGGCAGACCGTGTCTCAGGC GTTGGCTCTCATGGAGGTGG
SEQ ID NO: 278	SKI Converted DNA	TTCGGGTTACGGTTTTTTCGTTATTTTACGGGGCGGTTGTGGGTTTTTATTAGGTAGAGTCGTGTTTTTAGCGGT TGTTTTTATGGAGGTGG
SEQ ID NO: 279	SKI Forward Primer	ACGTTTTTTCGTTATTTTACGGG
SEQ ID NO: 280	SKI Reverse Primer	CAACGCTAAAAACACGACTC
SEQ ID NO: 281	SKI Flap oligonucleotide	CGCCGAGGGCGGTTGTGG/3C6/
SEQ ID NO: 282	SI1PR4 Target DNA	GGGCTGTCCGTTCCCTGCTCCCATACAGCGAGGTCGTGCACACAGCTTCTGTACCCAGGAGGCGCTGCC TGGCACGCAACCGGTGGCTGCACCATCCACACGCAAGACTGCAACTTCAGATGCTCCGCACGCTGGAGATG
SEQ ID NO: 283	SI1PR4 Converted DNA	GGGTTGTTTCGTTTTTGTTTTTATATAGCGGAGTTGCGTGTATATAGTTTTTGTATTTTAGGAGGGTTGTTTG GTAGGTATTCGGTGTGTTATTTATACGTAAGATTGTAATTTTAGATGTTTCGTACGTTGGAGATG
SEQ ID NO: 284	SI1PR4 Forward Primer	TTATATAGCGAGGTTGCGT
SEQ ID NO: 285	SI1PR4 Reverse Primer	CTTACGTATAATAATACAACCCGAATA
SEQ ID NO: 286	SI1PR4 Flap oligonucleotide	CCACGGACGACGTACCAACA/3C6/
SEQ ID NO: 287	SLC12A8 Target DNA	CGAGCTAGGAGGTTGGGCTCGGAGGGCGCAGGAAGACGGGCTCTCGAGGGAAGGGAAGAGAGGCCGCT TCTGGGAAGGGACCC

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:288	SLC12A8 Converted DNA	CGGAGTTAGGAGGGTGGGTTCCGAGGGCGTAGGAAGAGCGGTTTTTCGAGGAAAGGAGAGAGTCGTTTTTGGGAAGGGATT
SEQ ID NO:289	SLC12A8 Forward Primer	TTAGGAGGGTGGGTTCCG
SEQ ID NO:290	SLC12A8 Reverse Primer	CTTCTCGCAAAACCGC
SEQ ID NO:291	SLC12A8 Flap oligonucleotide	CCACGGACGGAGGGCGTAGG/3C6/
SEQ ID NO:292	SOBP Target DNA	GCCCCGGGGCCCCAGGCGCGCGGCTGCAACGTCATCGTGAACGGCACGGCGCG
SEQ ID NO:293	SOBP Converted DNA	GTTTCGGCGGGTTTCGAGGCGGTGCGGTTTGTAACTTATCGTGAACGGTACGCGCGG
SEQ ID NO:294	SOBP Forward Primer	TTTCGGCGGGTTTCGAG
SEQ ID NO:295	SOBP Reverse Primer	CGTACCGTTTACGATAACGT
SEQ ID NO:296	SOBP Flap oligonucleotide	CGCCGAGGGCGGTGCGGGT/3C6/
SEQ ID NO:297	SOBP Flap oligonucleotide v2	CGCCGAGGTTACAAACCGG/3C6/
SEQ ID NO:298	SPOCK2 Target DNA	CTAGGCGAGATGGTGGAAAGCGGTGTCGTACGGGGTGGGCTGGGTTCCCGTGCAGAAAGGCGCGGAGGACC CAGGCTGGTTTCCC
SEQ ID NO:299	SPOCK2 Converted DNA	TTAGGCGAGATGGTGGAAAGCGGTGTCGTACGGGGTGGGTTTGGGTTTTCGTGTAGAAGGGCGCGGAGGATTT AGTTGGTTTTTT
SEQ ID NO:300	SPOCK2 Forward Primer	CGAGATGGTGGAAAGCGG
SEQ ID NO:301	SPOCK2 Reverse Primer	GCSCCTTCTACAGAA
SEQ ID NO:302	SPOCK2 Flap oligonucleotide	CCACGGACGGTGTTCGTACGG/3C6/
SEQ ID NO:303	ST8SIA1 Target DNA	GGCTGTGCGCGCCAGCAAGGCGAGGGTCCGGAGAGGGTCCGCTCCTCTAAACATGTGGCCGTGGCG TCCCCTTGTCCTCCAGCGATGCTCCTGCGCCCTTCGCGCTCCGCGCTGCTGCGCCCGAGGCAA
SEQ ID NO:304	ST8SIA1 Converted DNA	GGCAGGGTCCGGAGAGGTTCCGTTTTTTTTAAATATGTGTTCTGTCGGCTTTTTTGTGTTTTTCGAGCGATGT TTTTGCGTTTTTCGTGTTTTTCGCGTTGTTGCGTCGTAGGTAA
SEQ ID NO:305	ST8SIA1 Forward Primer	AAATATGTGTTCTGTCGGCTT
SEQ ID NO:306	ST8SIA1 Reverse Primer	ACGCAACAACCGGAAAAAC
SEQ ID NO:307	ST8SIA1 Flap oligonucleotide	CGCCGAGGCGACGAAAAACG/3C6/
SEQ ID NO:308	ST8SIA1_22 Target DNA	ACGAGAAAGAGATCGTGCAGGGGGTGTGCAACAGGGCACGGCGTGGAGGAGAACAGACCGCGGCCAGAGC GTTAGGTACTCTGCTCCCTCCGCGGCTCCCTCTAGCGTCTTCTCCCGAGTGCAAGG
SEQ ID NO:309	ST8SIA1_22 Converted DNA	ACGAGAAAGAGATCGTGTAGGGGGTGTGTAATAGGGTACGGCTGGAGGAGGAATTAGATCGCGGTTAGAGCG TTTAGGTATTTTGTTCGCGGTTTTTTTTTAGCGTTTTTTTTTCGAGTGTAGAGG
SEQ ID NO:310	ST8SIA1_22 Forward Primer	GGGTGTGTAAATAGGTACG
SEQ ID NO:311	ST8SIA1_22 Reverse Primer	CTAAACGCTTAAACCGCA
SEQ ID NO:312	ST8SIA1_22 Flap oligonucleotide	CCACGGACGGCGTGGAGGAG/3C6/
SEQ ID NO:313	SP9 Target DNA	CGCCCGTTGTCACTCCGCGCCGACAGCGTCAATGGAAGCCCGACTTGTACCAGGACTCGTACGGGTGCGCC ATGCCACCGCGGGTACAGCCCGTCCGCTCGCTGTG
SEQ ID NO:314	SP9 Converted DNA	CGCGTCGTTGGTTATTCGTCGGTGTAGCGTCAATGGAAGTTCGATTGTATTAGGATTCGTACGGGTGCGTTA TGTTACGCGCGGGTATAGTTCGTCGGTTGTCGTCGTG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ.ID NO:315	SP9 Forward Primer	TAGCGTCGAATGGAAGTTCGA
SEQ.ID NO:316	SP9 Forward Primer: Universal	GGTCGTTAGCGTCGAATG
SEQ.ID NO:317	SP9 Reverse Primer	GC GCGTAAACATAACGCACC
SEQ.ID NO:318	SP9 Flap oligonucleotide	CCACGGACGCGTACGAATCC/3C6/
SEQ.ID NO:319	SUCLG2 Target DNA	GGTTCCTCCGCTGGGTTCTTAATCGTCGCTGACTTCAGAACTGCAGACCCTCGCGGTAAAGATGGCGGTGACACAGAA
SEQ.ID NO:320	SUCLG2 Converted DNA	GGTTTTTTTCGTGGGTTTTTAATCGTTCTGTTGATTTTTAGAATGAAATTGTAGATTTTCGCGGTAAAGATGGCGGTGA TAGAA
SEQ.ID NO:321	SUCLG2 Forward Primer	TCGTGGGTTTTTAATCGTTTCG
SEQ.ID NO:322	SUCLG2 Reverse Primer	TCAGGCCATCTTACCGC
SEQ.ID NO:323	SUCLG2 Flap oligonucleotide	CCACGGACGCGAAAATCTACA/3C6/
SEQ.ID NO:324	SUCLG2 Forward Primer: Universal	GGTTTTTTTCGTGGGTTTTTAATCG
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	GGAGTGAGTGCTACAAACGCGCAGCGCGGACTGATCCCCGTTGCTGCAGGTTGGTGCCCAAGCTGCGGGGTGCTC GGGCCCAACTAAAGCCAGCTCTGCCAGACGCGGAAAG
SEQ.ID NO:328	TBX15 Converted DNA	GGAGTGAGTGTTTATAACGCGTAGGTCGGATTGATTTTTCGTTGTTAGGTTGGTGTTTTAAGTTGCGGGGTGTTTCG GCGTTAATTAAAGTTAGTTTTGTTTAGACGCGGAAAG
SEQ.ID NO:329	TBX15 Reg. 1 Forward Primer	CGTAGGTCGGATTGATTTTTTCGT
SEQ.ID NO:330	TBX15 Reg. 1 Reverse Primer	TCTAAACAAAACTAACTTTAATTAACGCC
SEQ.ID NO:331	TBX15 Reg. 1 Flap oligonucleotide	CCACGGACGCGAACACCCGCA/3C6/
SEQ.ID NO:403	TBX15 Reg. 2 Target DNA	GGAAGGAAATTCGGGGTTCGGTCTCCGTTGCTCAGCTTCTGCTGAAGCCGCGTAGCAGTGAATGCGCGCTGA CTTTCAGCAGCACTCTCTGGAAGCAACGCCA
SEQ.ID NO:404	TBX15 Reg. 2 Converted DNA	GGAAGGAAATTCGGGGTTCGGTGTGTTGTTTGTGTTTGTAGTTTTTTGTTGAAGTTCGGTAGTAGTAATGCGCGTTGAT TTTTAGCGACGATTTTGGAAGTAAAGTTA
SEQ.ID NO:332	TBX15 Reg. 2 Forward Primer	AGGAAATGCGGGTTTTTCG
SEQ.ID NO:333	TBX15 Reg. 2 Forward Primer: Univ.	GGAAGGAAATTCGGGGTTTTTC
SEQ.ID NO:334	TBX15 Reg. 2 Reverse Primer	CCAAAAATCGTCGCTAAAAAATCAAC
SEQ.ID NO:335	TBX15 Reg. 2 Flap oligonucleotide	CCACGGACGCGGCATTCAC/3C6/
SEQ.ID NO:336	TRH Target DNA	GGCGGCCGCGACCCCTCCCGCTGACCTCACTCGAGCCGCCCTGCGGCAGATATAAGCGGCGGCCCATCTGAAG AGGGCTCGGACGCGCCCGGGGTC
SEQ.ID NO:337	TRH Converted DNA	GGCGTCCGCGATTTTTTTCGTTGATTTTATTCGAGTCGCTGTTTGGCGTAGATATAAGCGGCGGTTTTATTTTGAAGAG GGTTCGGTAGCGGTTTCGGGGTT
SEQ.ID NO:338	TRH Forward Primer	TTTCGTTGATTTTATTCGAGTCG
SEQ.ID NO:339	TRH Reverse Primer	TCTTCAAAATAAACCGCCGC
SEQ.ID NO:340	TRH Flap oligonucleotide	CGCCGAGGGTCGTTTGGCGT/3C6/

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:341	TSC22D4 Target DNA	CGGGTGGTGAAGTGCCTCCACGGCTGGAGAGCCTTATCGCCGGTCGCTGGACGTGTGGATGTTTATGAGC GAGACCTGGAGCCCAACAGCTTCGCGGACTCCTGGAGGAA
SEQ ID NO:342	TSC22D4 Converted DNA	CGGGTGGTGAAGTGTTCACGGTTGGAGAGTTTATCGTCGGTCTGGACGTGTGGATGTTTATGAGC GAGATTTGGAGTTTATAGTTTCGGCGGATTTTGGAGGAA
SEQ ID NO:343	TSC22D4 Forward Primer	GTTTGGAGAGTTTATCGTCG
SEQ ID NO:344	TSC22D4 Reverse Primer	CTCTCAAAATCCGCCGA
SEQ ID NO:345	TSC22D4 Flap oligonucleotide	CGCGAGGGCGTCTGTTGA/3C6/
SEQ ID NO:346	ZDHHC1 Target DNA	GGGCGGGGGCGACAGCCACGCTGGCGGCGAGGCGTGCGCCGCCGTTTCTGTGAGCCGAGCAG GGGTGCGGGTGCATAGTTTACGTTGGCGGTAGGCGCGTTCGTCGTTTCTGTGAGTTCGAGTAG
SEQ ID NO:347	ZDHHC1 Converted DNA	GTCCGGGTCGATAGTTTACG
SEQ ID NO:348	ZDHHC1 Forward Primer	ACTCGAATCACGAAACG
SEQ ID NO:349	ZDHHC1 Reverse Primer	CGCGAGGGACGAAACGACG/3C6/
SEQ ID NO:350	ZDHHC1 Flap oligonucleotide	GGAGCCCCAGCCCCACGCGGCGACACGAGGGTGGTGTACGCCCGCAGGGTCCGCGAGCGCGCGCAGAG
SEQ ID NO:351	ZMIZ1 Target DNA	CGCGGGCGTGGGAAGTTTCTC
SEQ ID NO:352	ZMIZ1 Converted DNA	GGAGTTTATAGTTTACGCGGTATACGTAGGGTGGTGGTTCGTTAGGTTTCGCGAGCGCGCGGTAGAGCG CGGGTCGTGGGAAGTTTTC
SEQ ID NO:353	ZMIZ1 Forward Primer	GTAGGTGGTGGTTACG
SEQ ID NO:354	ZMIZ1 Reverse Primer	AACTCCACGACCCGC
SEQ ID NO:355	ZMIZ1 Flap oligonucleotide	CGCGAGGGTTCGTAGGGTT/3C6/
SEQ ID NO:356	ZNF132 Target DNA	GGCGCCGCATTGCGGTCTCATTTTCTGCTGTTGGTGGTGGCTACAGCAGGCCCTCTGGAGCCACACCGGGCAC GGAGTGGGTGACGGGACCGTCACCGCGCTTCACACGACCATAGTCCCC
SEQ ID NO:357	ZNF132 Converted DNA	GGCGTGTATTGCGGTTTATTTTGTGTTGGTGGTGGTATAGGTTTGGAGTTATATTAGGGTACGG GAGTGGGTGTAAGGATCGTTATCGCGTTTATACGTATTATAGTGTTC
SEQ ID NO:358	ZNF132 Forward Primer	TGGAGTTATTAGGGTACGGGA
SEQ ID NO:359	ZNF132 Reverse Primer	ACACTATAATACGTATAAAACGCGATA
SEQ ID NO:360	ZNF132 Flap oligonucleotide	CCACGGACGAACGATCCCTAC/3C6/
SEQ ID NO:361	ZNF329 Target DNA	GGCGCGAGGGGCGCGTCCGCGGGTGGGTTTACCTGGGTGGTGGGCTGCGGCCCTAGGGCGAGGGTCT GGCAGGGGCGTAGTTCTCTGTGGTGGGACGCTCCGTGGCGATTGGGGTCACTCCTCTGAGG
SEQ ID NO:362	ZNF329 Converted DNA	GGCGCGAGGGGCGCGTCCGCGGGTGGGTTTATTTGGGTGGGTATGTCGGGTTCTGTTAGGGCGAGGGTTTG GTTAGGGGCGTAGTTTTCGTTGGTGGGACGTTTCGTGGGATTGGGGTTATTTTTTTGAGG
SEQ ID NO:363	ZNF329 Forward Primer	GGTGGTGGGTATGTCGG
SEQ ID NO:364	ZNF329 Reverse Primer	CCAATCGCCACGAAACG
SEQ ID NO:365	ZNF329 Flap oligonucleotide	CCACGGACGGTTCGTTAGGG/3C6/
SEQ ID NO:366	ZNF671 Target DNA	CCGTGGCGCGACAGCTGCCGGGAGCGGCGGCTCTCGATCGGGGACGACGACCTCCGTCCTCAGAGCA TCAGACGCGTCTCGGACACTGGGACCAACATCTCTCCCGC
SEQ ID NO:367	ZNF671 Converted DNA	TCGTGGCGCGGATAGTTGTCGGGAGCGGTTCGATCGGGGACGTAGGTATTTTCGTTTTTGTAGAGTAT TAGACGCGTTTCGGGATATTGGGATAATAATTTTTTCGCG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:368	ZNF671 Forward Primer	GTGTGGGAGCGGTAGG
SEQ ID NO:369	ZNF671 Reverse Primer	CCAATATCCCGAAACGCGTCT
SEQ ID NO:370	ZNF671 Flap oligonucleotide	CCACGGACGGCGTTTCGATCG/3C6/
SEQ ID NO:371	ZNF781 Target DNA	AAGCTGCCGCCGGAGACGTGGGAGCGTTCTCTTGTTCAGTGCGCGGACTCATCGGGTCACAGTTTATGCTTTT ATGACGCGGTGAGTCCAGCCACTGATTCCTAACGGTTTAGAGT
SEQ ID NO:372	ZNF781 Converted DNA	AAGTTCGTTCCGGAGACGTGGGAGCGTTTCTTGTTCAGTGCGCGGATTTATCGGGTTATAGTTTATGTTTTTA TGACGCGGTGAGTTAGTTATTGATTTTAAACGGTTTAGAGT
SEQ ID NO:373	ZNF781 Forward Primer	CGTTTTTTGTTTTCGAGTGCG
SEQ ID NO:374	ZNF781 Reverse Primer	TCAATAACTAACTCACCGCGTC
SEQ ID NO:375	ZNF781 Flap oligonucleotide	CCACGGACGGCGGATTATTCG/3C6/
SEQ ID NO:376	β -actin Target DNA (ACTB)	CTCTGACCTGAGTCTCTTTGGAACTCTGCAGGTTCTATTGCTTTTCCAGATGAGCTCTTTTCTGGTGTGTCTC TCTGACTAGGTGCTAAGACAGTGTTGTGGGTAGGTACTAACACTGGCTGTGTGACAAAGGCCATGAGGCTGGT GTAAGCGGCCCTTGGAGTGTATTAAAGTAGGTGCACAGTAGGTCTGAACAGACTCCCATCCCAAGA
SEQ ID NO:377	β -actin Converted (BTACT)	TTTTGATTGAGTTTTTTTGGAAATTTGTAGGTTTATTGTTTTTTTATAGTAGGTTTTTTTTTGGTGTGTTTTT TGATTAGGTGTTAAGATAGTGTGTGGGTAGGTATTAAATATGTTGTGTGATAGGTTATGAGGTTGGTGTGA AAGTGGTTTGGAGTGTATTAAAGTAGGTGTATAGTAGGTTTGAATAGATTTTTTATTAAAGA
SEQ ID NO:378	β -actin UT Forward primer	CCATGAGGCTGGGTAAAG
SEQ ID NO:379	β -actin UT Reverse primer	CTACTGTGCACCTACTTAATACAC
SEQ ID NO:380	β -actin UT Probe (Arm 1)	CGCCGAGGGCGGCCCTGGAG/3C6/
SEQ ID NO:381	β -actin BT Forward primer 65	GTGTTTGTTTTTTATTAGGTGTTTAAAGA
SEQ ID NO:382	β -actin BT Reverse primer 65	CTTTACCAACCTCATAACCTTATC
SEQ ID NO:383	β -actin BT probe (Arm 3)	GACGCGGAGATAGTGTGTGG/3C6/
SEQ ID NO:384	B3GALT6 Target DNA	GGCCACACAGGCCCACTCTGGCCCTCTGAGCCCCGGGACCCAGGGCATTCAAGGAGCGGCTCTGGGCTGCCA GCGCAGGCCCTCCGCGCAAAACACAGCAGGCTGGAAGTGGCGCTCATCACCGGCACGCTCTCCAG
SEQ ID NO:385	B3GALT6 Converted DNA	GGTTATAGGTTTATTGTTGTTTTTGTAGTTTTCGGCGGATTAGGTTTAAAGGAGCGGTTTTTGGGTTGTTAGCG TAGGTTTTCGCGTAAATATAGTAGGTTGGAAGTGGCGTTTATTATCGGTACGTTTTTTTAG
SEQ ID NO:386	B3GALT6 Forward Primer	GGTTATTGTTGTTTTGAGTTTTCGG
SEQ ID NO:387	B3GALT6 Reverse Primer	TCCAACCTACTATATTACGCGAA
SEQ ID NO:388	B3GALT6 Flap oligonucleotide (Arm 5)	CCACGGACGGCGGATTTAGGG/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/GTGTAACCAACAGGA/IME- dC/GAGGACATGACTTTCAGCCCTGCAGCCAGACACAGCTGAGCTGGTGTGACCTGTGTGGAGAGTTCATCTGG

FIG. 6 (cont'd)

	Description	Sequence (all are shown 5' to 3')
SEQ ID NO:390	Zebrafish (ZF) Convert. Synthetic Target DNA	TTTATCGTGGTGTATTATTTGGATAGGTGGAGTAGAGGGAAGGTGGTCCGTATGGTGGCGAGCGCGTTCGTTTGG AGGATTTTCGATTGGTTGACGTGTAAATTAGGACGAGGATATGATTTTAGTTTGTAGTTAGATATAGTTGAGTTGG TGTGATTTGTGTGGAGAGTTTATTGG
SEQ ID NO:391	ZF_RASSF1 UT Forward primer	CGCATGGTGGCGGAG
SEQ ID NO:392	ZF_RASSF1 UT Reverse primer	ACACGTCAGCCAATCGGG
SEQ ID NO:393	ZF_RASSF1 UT Probe (Arm 3)	GACGCGGAGGCGCGTGGCC/3C6/
SEQ ID NO:394	ZF_RASSF1 BT Forward primer	TGCGTATGGTGGCGGAG
SEQ ID NO:395	ZF_RASSF1 BT Reverse primer	CCTAATTTACACGTCAACCAATCGAA
SEQ ID NO:396	ZF_RASSF1 BT probe (Arm 3)	GACGCGGAGGCGCGTGGCTTT/3C6/
SEQ ID NO:397	ZF_RASSF1 BT probe (Arm 5)	CCACGGACGGCGCGTGGCTTT/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. 7

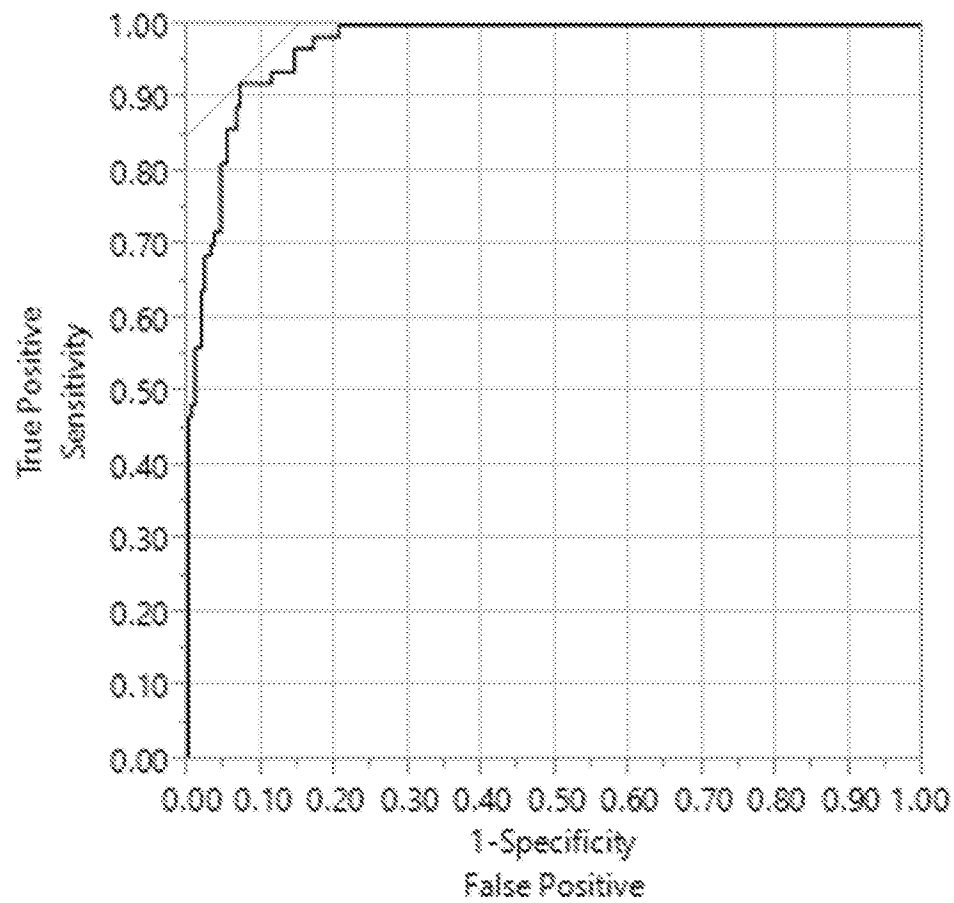
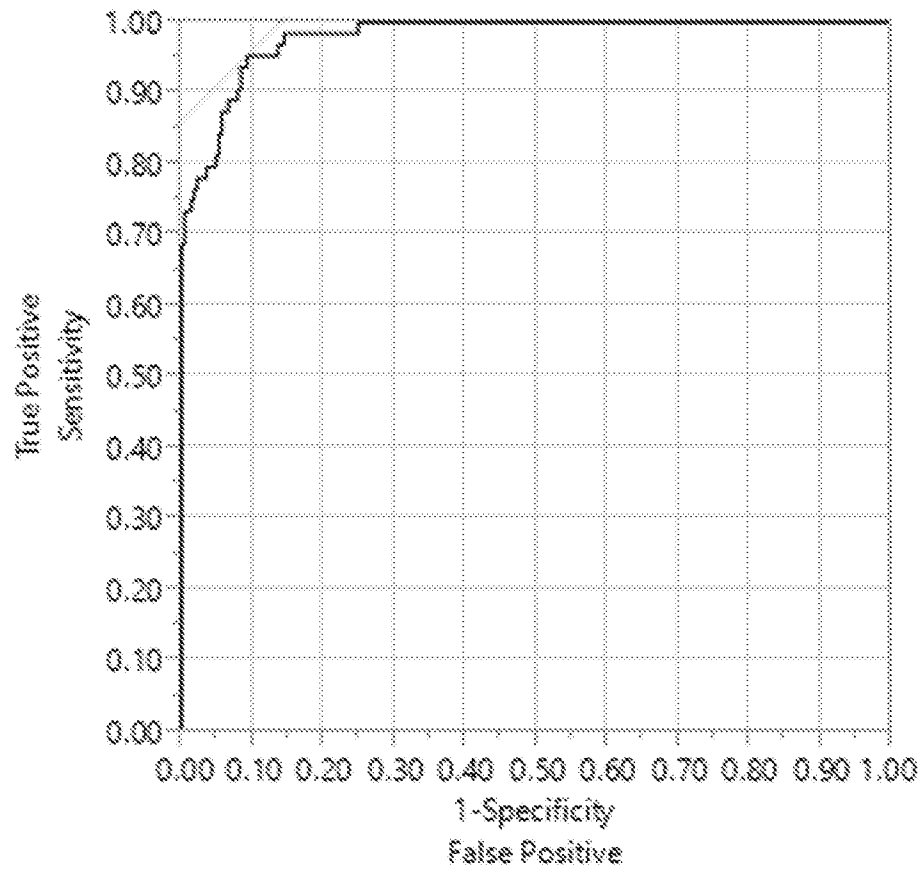


FIG. 8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24468

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C12Q 1/68 (2017.01)

CPC - C12Q 1/6886, C12Q 2600/106, C12Q 2600/154, C12Q 2600/112, C12Q 1/6827, C12Q 2600/172, C12Q 1/6883, C12Q 2537/164

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

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

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	Carvalho, et al. Genome-wide DNA methylation profiling of non-small cell lung carcinomas. Epigenetics & Chromatin 2012, 5:9; pg 12, Table 3; pg 15	1-3, 7, 25-28 ----- 18
X	Kober, et al. Methyl-CpG binding column-based identification of nine genes hypermethylated in colorectal cancer. Mol Carcinog. 2011, 50(11):846-56; Supplementary Material, pg 2, Fig 2; pg 7, Table 4	38-40
Y	Ponomaryova, et al. Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients. Lung Cancer 2013, 81(3):397-403; pg 398; pg 401, col 2	17, 18
Y	US 2016/0010081 A1 (Exact Sciences Corporation) 14 January 2016 (14.01.2016) claims 1, 10-14; para [0208], [0209]	17, 18

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

13 June 2017

Date of mailing of the international search report

01-SEP 2017

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24468

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-6, 8-16, 19-24, 29-37, 41-47
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

***** See Supplemental Sheet to continue *****

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-3, 7, 17, 18, 25-28, 38-40, restricted to BARX1

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24468

In Continuation of Box III. Observations where unity of invention is lacking:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

Group I+: claims 1-3, 7, 17, 18, 25-28, 38-40, directed to a method of processing a sample/analyzing a plasma sample, a kit, and a composition. The method, kit, and the composition will be searched to the extent that a methylation marker or target nucleic acid encompasses BARX1. It is believed that claims 1-3, 7, 17, 18, 25-28, 38-40 encompass this first named invention, and thus these claims will be searched without fee to the extent that they encompass a method of processing a sample/analyzing a plasma sample for the presence of methylated BARX1, a kit comprising an oligonucleotide specifically hybridizing to a BARX1 methylation marker, and a composition comprising a complex of BARX1 target nucleic acid, and an oligonucleotide that specifically hybridizes to said BARX1 target nucleic acid. Additional methylation marker(s)/target nucleic acid(s) will be searched upon the payment of additional fees. Applicants must specify the claims that encompass any additionally elected methylation marker(s)/target nucleic acid(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched. An exemplary election would be a method of processing a sample/analyzing a plasma sample for the presence of methylated ZNF329, a kit comprising an oligonucleotide specifically hybridizing to a ZNF329 methylation marker, and a composition comprising a complex of ZNF329 target nucleic acid, and an oligonucleotide that specifically hybridizes to said ZNF329 target nucleic acid, i.e., claims 1-3, 7, 17, 18, 25-28, 38-40.

The inventions listed as Group I+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

Special Technical Features

The special technical feature of each invention of Group I+ is a specific methylation marker/target nucleic acid recited therein.

Common Technical Features

Some inventions of Group I+ share the technical feature of a method of processing a sample by a) assaying a sample from a subject for an amount of at least one methylation marker, b) assaying said sample for an amount of a reference marker; c) comparing the amount of said at least one 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. Some inventions of Group I+ share the technical feature of a composition comprising a complex of a target nucleic acid, and an oligonucleotide that specifically hybridizes to said target nucleic acid. However, these shared technical features do not represent a contribution over prior art as being anticipated by a paper titled "DNA methylation of the homeobox genes PITX2 and SHOX2 predicts outcome in non-small-cell lung cancer patients" by Dietrich, et al. (Diagn Mol Pathol. 2012, 21(2):93-104) (hereinafter "Dietrich").

Dietrich discloses a method of processing a sample (pg 94, col 2, "Preparation of Sample DNA"; pg 95, col 1, "Real-time PCR Quantification of SHOX2 and PITX2 DNA Methylation"), the method comprising:

a) assaying a sample from a subject for an amount of at least one methylation marker (Abstract, "the ability of the DNA methylation biomarkers PITX2 and SHOX2 to predict disease outcome in lung cancer patients has been evaluated. Quantitative, methylation-specific (HeavyMethyl), real-time polymerase chain reaction assays were used to measure DNA methylation of PITX2 and SHOX2 in bisulfite-converted DNA from formalin-fixed, paraffin-embedded tissues from 474 non-small-cell lung cancer patients"),
b) assaying said sample for an amount of a reference marker and c) comparing the amount of said at least one methylation marker to the amount of reference marker in said sample (pg 95, col 1, "Relative DNA methylation of the SHOX2 locus as compared with total DNA (determined using ACTB reference in a duplex PCR reaction)... Relative DNA methylation of the PITX2 locus referred to total DNA as determined with a methylation unspecific PITX2 assay (PITX2reference) was analyzed"; pg 97, "FIGURE 1. Distribution (histogram) of (B) SHOX2 and (A) PITX2 DNA methylation among the analyzed samples from non-small cell lung cancer patients")
to determine a methylation state for said at least one methylation marker in said sample (pg 95, col 2, "For each sample, a relative methylation value was determined using the [delta][delta]CT method...").

Dietrich discloses a composition comprising a complex of a target nucleic acid and an oligonucleotide that specifically hybridizes to said target nucleic acid (pg 95, col 1, "Relative DNA methylation of the SHOX2 locus as compared with total DNA ... was quantified in 20 mL PCR reaction with the following composition: ... primers (SHOX2: 0.4 mM gtttttgatagtttagta, 0.4 mM cctcctaccttctaacc;... 0.3 mM each detection probe (SHOX2: 6-FAM-ctcgtacgaccccgatcg-BBQ-650..."). As said technical features were known in the art at the time of the invention, these cannot be considered special technical feature that would otherwise unify the inventions.

Some inventions of Group I+ share the technical feature of a kit, comprising: a) at least one oligonucleotide, wherein at least a portion of said oligonucleotide specifically hybridizes to a methylation marker, b) at least one additional oligonucleotide, wherein at least a portion of said additional oligonucleotide specifically hybridizes to a reference nucleic acid. However, these shared technical features do not represent a contribution over prior art as being anticipated by US 2016/0017435 A1 to HUDSONALPHA INSTITUTE FOR BIOTECHNOLOGY, et al. (21 January 2016) (hereinafter "HUDSONALPHA").

HUDSONALPHA discloses such a kit (claim 15, "A kit for determining the presence or absence of the biochemical reoccurrence of prostate cancer in an individual, the kit comprising: a. a plurality of nucleic acid primers configured to bind to a nucleic acid at least 90% identical to a sequence selected from the group consisting of SEQ ID NOS.: 1-75; b. wherein the primers are for use in a polymerase chain reaction (PCR) reaction; wherein the primers are configured to aid in the determination of the methylation level of at least one cytosine within the nucleic acid"). As said technical feature was known in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions.

***** See the Following Supplemental Sheet to continue *****

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 17/24468

In Continuation of Box III. Observations where unity of invention is lacking:

Some inventions of Group I+ share the technical feature of a method of analyzing a plasma sample obtained from a subject suspected of having a lung neoplasm, the method comprising:

a) combining the plasma sample with i) protease; and ii) a first lysis reagent b) to the mixture of step a) adding iii) silica particles, and iv) a second lysis reagent; c) separating silica particles with bound DNA from the mixture of b); d) to the separated silica particles with bound DNA adding a first wash solution, said first wash solution; e) separating the silica particles with bound DNA from said first wash solution; f) to the separated silica particles with bound DNA adding a second wash solution; g) separating washed silica particles with bound DNA from said second wash solution; and h) eluting DNA from the washed silica particles with bound DNA separated in step g); i) assaying eluted DNA for an amount of at least one methylated methylation marker and for an amount of reference marker in said eluted DNA; j) comparing the amount of said at least one methylated methylation marker to the amount of reference marker in said DNA to determine a methylation state for said at least one methylation marker in said plasma sample. However, this shared technical feature does not represent a contribution over prior art as being obvious over a paper titled "Potentialities of aberrantly methylated circulating DNA for diagnostics and post-treatment follow-up of lung cancer patients" by Ponomaryova, et al. (Lung Cancer 2013, 81(3):397-403) (hereinafter "Ponomaryova") in view of US 2016/0010081 A1 to Exact Sciences Corporation (14 January 2016) (hereinafter "Exact Sciences").

Ponomaryova discloses a method of analyzing a plasma sample obtained from a subject suspected of having a lung neoplasm (pg 401, col 2, "the accuracy of differentiating lung cancer patients from healthy subjects was higher using the two-marker combination than using each marker alone. Random forest classification tree model estimated high importance score for plasma RARB2 IM, moderate for csb RARB2 IM, plasma RASSF1A IM, csb RASSF1A IM variables and low for a patient age variable. Machine learning model based on these variables combined lead to NSCLC patients... and healthy subjects... differentiation with 87% sensitivity and 75% specificity"), the method comprising:

a) combining the plasma sample with i) protease (pg 398, col 1, "2.2. Preparation of plasma and cell-surface-bound fractions, DNA extraction and bisulfite treatment. Venous blood samples were stabilized and fractionated into plasma and blood cells The csb-cirDNA fractions were obtained as previously described [15]. Briefly, blood cells were incubated with 9 volumes of phosphate buffered saline (PBS) containing 5 mM EDTA (PBS/EDTA) (5 min, room temperature). The cells were pelleted by centrifugation and incubated with an equal volume of 0.25% trypsin solution").

Ponomaryova discloses that "cirDNA was isolated from 1 mL of plasma, 3 mL of PBS/EDTA and 1 mL of trypsin eluates and modified with sodium bisulfite, followed by purification using a blood DNA isolation kit (BioSilica Ltd., Novosibirsk, Russia)", but does not specifically disclose ii) a first lysis reagent, said first lysis reagent comprising guanidine thiocyanate; and non-ionic detergent; to form a mixture wherein proteins are digested by said protease; b) to the mixture of step a) adding iii) silica particles, and iv) a second lysis reagent, said second lysis reagent comprising: guanidine thiocyanate; non-ionic detergent; and isopropyl alcohol; under conditions wherein DNA is bound to said silica particles; c) separating silica particles with bound DNA from the mixture of b); d) to the separated silica particles with bound DNA adding a first wash solution, said first wash solution comprising A) guanidine hydrochloride or guanidine thiocyanate, and B) ethyl alcohol; e) separating the silica particles with bound DNA from said first wash solution; f) to the separated silica particles with bound DNA adding a second wash solution, said second wash solution comprising a buffer and ethyl alcohol; g) separating washed silica particles with bound DNA from said second wash solution; and h) eluting DNA from the washed silica particles with bound DNA separated in step g).

Ponomaryova further discloses i) assaying eluted DNA for an amount of at least one methylated methylation marker and for an amount of reference marker in said eluted DNA; j) comparing the amount of said at least one methylated methylation marker to the amount of reference marker in said DNA to determine a methylation state for said at least one methylation marker in said plasma sample (pg 398, col 1 to col 2, "2.3. Measurement of tumor suppressor gene methylation levels in cirDNA. Concentrations of methylated and unmethylated forms of the RASSF1A gene were assessed by quantitative methylation-specific TaqMan PCR (MSP). Concentrations of methylated and unmethylated forms of the RARB2 gene were assessed by EvaGreen PCR (Biotium, Hayward, California, USA)... Standard curves were generated using serial dilutions of the purified methylated and unmethylated MSP amplification products... The index of gene methylation (IM) was calculated as % IM = 100*[copy numbers of methylated gene/(copy numbers of methylated gene + unmethylated gene)]").

Exact Sciences discloses, in the context of "[a] method of treating a sample of stimulated pancreatic juice from a subject" (Exact Sciences, claim 1, see also claim 10), a method comprising a) combining the pancreatic juice sample with: i) protease; and ii) a first lysis reagent, said first lysis reagent comprising guanidine thiocyanate; and non-ionic detergent; to form a mixture wherein proteins are digested by said protease iv) a second lysis reagent, said second lysis reagent comprising: guanidine thiocyanate; non-ionic detergent; and isopropyl alcohol; under conditions wherein DNA is bound to said silica particles (Exact Sciences, claim 10, "A method of assaying DNA in a sample of stimulated pancreatic juice from a subject, the method comprising: a) treating said sample with proteinase K; b) combining the sample treated with proteinase K with a lysis reagent comprising guanidine HCl and polyoxyethylene (20) sorbitan monolaurate, and with isopropanol to form a sample mixture, c) adding mixable particles to said sample mixture under conditions wherein DNA in the sample binds to said mixable particles..."), and

c) separating silica particles with bound DNA from the mixture of b); d) to the separated silica particles with bound DNA adding a first wash solution, said first wash solution comprising A) guanidine hydrochloride or guanidine thiocyanate, and B) ethyl alcohol; e) separating the silica particles with bound DNA from said first wash solution; f) to the separated silica particles with bound DNA adding a second wash solution, said second wash solution comprising a buffer and ethyl alcohol; g) separating washed silica particles with bound DNA from said second wash solution; and h) eluting DNA from the washed silica particles with bound DNA separated in step g); i) assaying eluted DNA for an amount of at least one methylated methylation marker and for an amount of reference marker in said eluted DNA (Exact Sciences, claims 10-14, "d) separating mixable particles bound to DNA from said sample mixture; e) washing said mixable particles bound to DNA; f) extracting bound DNA from said mixable particles; and g) performing a nucleic acid detection assay comprising DNA extracted from said mixable particles. 12. The method of claim 10, wherein said lysis reagent comprises about 7M guanidine HCl and 20% polyoxyethylene (20) sorbitan monolaurate. 13. The method of claim 10, wherein said washing comprises exposing said mixable particles bound to DNA to a wash buffer comprising Tris-HCl and ethanol. 14. The method claim 10, wherein said extracting said bound DNA from said mixable particles comprises exposing said mixable particles bound to DNA to an elution buffer comprising Tris-HCl and EDTA");

***** See the Following Supplemental Sheet to continue *****

In Continuation of Box III. Observations where unity of invention is lacking and the Preceding Supplemental Sheet:

j) comparing the amount of said at least one methylated methylation marker to the amount of reference marker in said DNA to determine a methylation state for said at least one methylation marker in said plasma sample (Exact Sciences, para [0208]-[0209], "Example 7. Sensitive DNA Marker Panel for Detection of Pancreatic Cancer by Assay of DNA in Stimulated Pancreatic Juice... [0209] Experiments are performed to compare methylated DNA markers and mutant KRAS assayed alone and combined in stimulated pancreatic juice to distinguish pancreatic cancer (PanC) samples from chronic pancreatitis (CP) and reference controls (CON)"). It would have been obvious to one of ordinary skill in the art to combine, in the course of routine experimentation and with a reasonable expectation of success, Ponomaryova and Exact Sciences, by incorporating steps a) ii) through h) disclosed by Exact Sciences into the method of analyzing a plasma sample obtained from a subject suspected of having a lung neoplasm disclosed by Ponomaryova, to improve purity of resulting purified DNA. As said technical feature would have been obvious to one of ordinary skill in the art at the time of the invention, this cannot be considered special technical feature that would otherwise unify the inventions.

The inventions of Group I+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.