

WHAT CLAIMED IS:

1. A method of diagnosing colorectal neoplasm, screening for the onset or risk to the onset of colorectal neoplasm or assessing the development or prognosis of colorectal neoplasm in a subject, said method comprises the following steps:
 - (I). treating a DNA obtained from a biological sample with a reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA, thereby obtaining a treated DNA;
 - (II). quantifying individual methylation level of a set of target markers within the treated DNA of step (I), wherein the target markers are selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; and
 - (III). comparing the methylation level of at least one target marker of the set of target markers quantified at step (II) respectively with a corresponding reference level, wherein an identical or higher methylation level of one or more of the target markers relative to its corresponding reference level indicates that the subject has colorectal neoplasm, or is at the onset or at a risk to the onset of colorectal neoplasm, or develops or with an increased probability of developing colorectal neoplasm, or has poor prognosis or at a risk to poor prognosis of colorectal neoplasm.
2. A method of diagnosing colorectal neoplasm, screening for the onset or risk to the onset of colorectal neoplasm or assessing the development or prognosis of colorectal neoplasm in a subject, said method ~~comprising~~ comprises the following steps:
 - (I). treating a DNA obtained from a biological sample with a reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA, thereby obtaining a treated DNA;
 - (II). quantifying individual methylation level of a set of target markers within the treated DNA of step (I), wherein at least two target markers are selected from the

group consisting of Septin9, BCAT1, IKZF1, BCAN, PKNOX2, VAV3, NDRG4 and IRF4, and at least two target markers are selected from the group consisting of POU4F2, SALL1, SDC2, ASCL4, INTERGENIC REGION 1, TMEFF2, INTERGENIC REGION 4, NKX2-6, INTERGENIC REGION 5, SLC24A2, INTERGENIC REGION 2, INTERGENIC REGION 3, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, and CRHBP, and

(III). comparing the methylation level of at least one target marker of the set of target markers quantified at step (II) respectively with a corresponding reference level, wherein an identical or higher methylation level of one or more of the target markers relative to its corresponding reference level indicates that the subject has colorectal neoplasm, or is at the onset or at a risk to the onset of colorectal neoplasm, or develops or with an increased probability of developing colorectal neoplasm, or has poor prognosis or at a risk to poor prognosis of colorectal neoplasm.

~~3. The method of claim 1 or 2, wherein the set of target markers comprises four, five, six, seven, eight, nine or more target markers.~~

~~4.~~ 3. The method of claim 1 or 2, wherein the step (II) comprises:

(i) pre-amplifying at least a portion of at least one target marker of a set of target markers within the treated DNA obtained from step (I) with a pre-amplification primer pool, and the set of target markers are selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; and

(ii) quantifying individual methylation level of the set of target markers within achieved DNA from the said sub-step (i).

~~5. The method of claim 1 or 2, further comprising obtaining DNA from a biological sample from a subject before the step (I).~~

~~6.~~ 4. A method of diagnosing colorectal neoplasm, screening for the onset or risk to the onset of colorectal neoplasm or assessing the development or prognosis of colorectal neoplasm in a subject, said method comprises the following steps:

- (a). obtaining a biological sample containing DNA from the subject;
- (b). treating the DNA in the biological sample obtained from step (a) with a reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA, thereby obtaining a treated DNA;
- (c). pre-amplifying at least a portion of at least one target marker within the treated DNA obtained from step (b) with a pre-amplification primer pool, wherein at least a portion of at least one of the target marker(s) is pre-amplified, and the at least one target marker comprises one or more markers selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; wherein step (c) is present or absent;
- (d). if step (c) is present, then quantifying individually methylation level of the at least one target marker based on achieved DNA from step (c); if step (c) is absent, then quantifying individually methylation level of at least one target marker within the treated DNA obtained from step (b), wherein the at least one target marker comprises one or more markers selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; and
- (e). comparing the methylation level of at least one target marker from step (d) respectively with a corresponding reference level, wherein an identical or higher methylation level of one or more of the target

marker(s) relative to its corresponding reference level indicates that the subject has colorectal neoplasm, or is at the onset or at a risk to the onset of colorectal neoplasm, or develops or with an increased probability of developing colorectal neoplasm, or has poor prognosis or at a risk to poor prognosis of colorectal neoplasm.

7. 5. A method of monitoring treatment response in a subject who is receiving treatment of colorectal neoplasm, comprising the following steps:
- (a). obtaining a biological sample containing DNA from the subject;
 - (b). treating the DNA in the biological sample obtained from step (a) with a reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA, thereby obtaining a treated DNA;
 - (c). pre-amplifying at least a portion of at least one target marker within the treated DNA obtained from step (b) with a pre-amplification primer pool, wherein at least a portion of at least one of the target marker(s) is pre-amplified, and the at least one target marker comprises one or more markers selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; wherein step (c) is present or absent;
 - (d). if step (c) is present, then quantifying individually methylation level of the at least one target marker based on achieved DNA from step (c); if step (c) is absent, then quantifying individually methylation level of at least one target marker within the treated DNA obtained from step (b), wherein the at least one target marker comprises one or more markers selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1,

INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5; and

- (e). comparing the methylation level of at least one target marker from step (d) respectively with a corresponding methylation level of one or more of the target marker(s) obtained from the same subject prior to the treatment which is quantified by repeating step (a), step (b), optionally step (c), and step (d) with respect to a biological sample containing DNA obtained from the subject prior to the treatment, wherein a lower methylation level of one or more of the target marker(s) relative to its corresponding methylation level prior to the treatment indicates that the subject is responsive to the treatment.

~~8. 6.~~ The method of claim ~~6~~ 4, wherein the at least one target marker comprises multiple target markers, wherein the multiple target markers comprise at least two markers selected from the group consisting of Septin9, BCAT1, and IKZF1; preferably wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of BCAN, PKNOX2, VAV3, NDRG4, and IRF4; preferably wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of POU4F2, SALL1, SDC2, ASCL4, INTERGENIC REGION 1, TMEFF2, INTERGENIC REGION 4, NKX2-6, INTERGENIC REGION 5, SLC24A2, INTERGENIC REGION 2, INTERGENIC REGION 3, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, and CRHBP.

~~9. The method of claim 7, wherein the at least one target marker comprises multiple target markers, wherein the multiple target markers comprise at least two markers selected from the group consisting of Septin9, BCAT1, and IKZF1.~~

~~10. The method of claim 8 or 9, wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of BCAN, PKNOX2, VAV3, NDRG4, and IRF4.~~

~~11. The method of claim 10, wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of POU4F2, SALL1, SDC2, ASCL4, INTERGENIC REGION 1, TMEFF2, INTERGENIC REGION 4, NKX2-6, INTERGENIC REGION 5, SLC24A2, INTERGENIC~~

~~REGION 2, INTERGENIC REGION 3, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, and CRHBP.~~

- ~~12.~~ 7. The method of any one of claims 1-~~11~~ 6, wherein the respective target marker comprises or is: a) the respective region defined by Hg19 coordinates as set forth below:

Target Marker	Hg19 Coordinate
NDRG4	chr16:58496750-58547532
BCAT1	chr12:24964295-25102393
IKZF1	chr7:50343720-50472799
Septin9	chr17:75276651-75496678
SDC2	chr8:97505579-97624000
VAV3	chr1:108113782-108507766
IRF4	chr6:391739-411447
TMEFF2	chr2:192813769-193060435
SALL1	chr16:51169886-51185278
BCAN	chr1:156611182-156629324
POU4F2	chr4:147560045-147563626
PKNOX2	chr11:125034583-125303285
ASCL4	chr12:108168162-108170421
KCNA6	chr12:4918342-4960277
SOX1	chr13:112721913-112726020
HS3ST2	chr16:22825498-22927659
FGF12	chr3:191857184-192485553
KCTD8	chr4:44175926-44450824

HMX1	chr4:8847802-8873543
MARCH11	chr5:16067248-16180871
CRHBP	chr5:76248538-76276983
NKX2-6	chr8:23559964-23564111
SLC24A2	chr9:19507450-19786926
INTERGENIC REGION 1	chr6:19679885-19693988
INTERGENIC REGION 2	chr10:130082033-130087148
INTERGENIC REGION 3	chr10:133107880-133113966
INTERGENIC REGION 4	chr7:152620588-152624685
INTERGENIC REGION 5	chr8:70945014-70949177

, and 5 kb upstream of the respective start site and 5 kb downstream of the respective end site of each region described above, or b) a bisulfite converted counterpart of a), or c) a MSRE treated counterpart of a).

~~13.~~ 8. The method of any one of the preceding claims, wherein the DNA comprises genomic DNA or cell-free DNA.

~~14.~~ 9. The method of claim ~~13~~ 8, wherein the cell-free DNA comprises circulating tumor DNA.

~~15.~~ 10. The method of claim ~~13~~ 8 or ~~14~~ 9, wherein the target marker in the cell-free DNA is present in the biological sample in an amount no more than 1ng, 0.8ng, 0.6ng, 0.4ng, 0.2 ng, 0.1ng, 0.08 ng or no more than 0.04ng.

~~16.~~ The method of claim ~~15~~, wherein the target marker in the cell-free DNA is present in the biological sample at a concentration that is below a level of sensitivity of a detection assay for the target marker.

~~17.~~ The method of any one of the preceding claims, wherein the achieved DNA from sub-step (i) or step (c) is diluted with a diluent prior to sub-step (ii) or step (d).

~~18.~~ 11. The method of any one of the preceding claims, wherein the biological sample is selected from the group consisting of a tissue section, biopsy, a

paraffin-embedded tissue, a body fluid, colonic effluent, a surgical resection sample, an isolated blood cell, a cell isolated from blood, and any combination thereof; preferably wherein the body fluid is selected from the group consisting of whole blood, blood serum, blood plasma, urine, mucus, saliva, peritoneal fluid, pleural fluid, chest fluid, synovial fluid, cerebrospinal fluid, thoracentesis fluid, abdominal fluid, and any combination thereof.

- ~~19. The method of claim 18, wherein the body fluid is selected from the group consisting of whole blood, blood serum, blood plasma, urine, mucus, saliva, peritoneal fluid, pleural fluid, chest fluid, synovial fluid, cerebrospinal fluid, thoracentesis fluid, abdominal fluid, and any combination thereof.—~~
- ~~20. The method of claim 19, wherein the biological sample is obtained from blood plasma of the subject.—~~
- ~~21. The method of claim 18, wherein the colonic effluent is selected from the group consisting of a stool sample and an enema wash sample.—~~
22. 12. The method of any one of the preceding claims, wherein the reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA selectively modifies at unmethylated cytosine residue(s) at the CpG site(s) to produce modified residue(s) but does not significantly modify methylated cytosine residue(s).
- ~~23. The method of claim 22, wherein the reagent comprises a bisulfite reagent.~~
- ~~24. The method of claim 23, wherein the bisulfite reagent is selected from the group consisting of ammonium bisulfite, sodium bisulfite, potassium bisulfite, calcium bisulfite, magnesium bisulfite, aluminum bisulfite, hydrogen sulfite and any combination thereof.—~~
- ~~25. The method of any one of claims 1-24, wherein the reagent capable of distinguishing between an unmethylated site and a methylated site in the DNA selectively cleaves at a residue when it is unmethylated but does not cleave at the residue when it is methylated, or selectively cleaves at the residue when it is methylated but does not cleave at the residue when it is unmethylated.~~
- ~~26. The method of claim 25, wherein the reagent is a methylation sensitive restriction enzyme (MSRE).—~~

- ~~27. The method of claim 26, wherein the MSRE is selected from the group consisting of *HpaII*, *SaII*, *SaII* HF[®], *ScrFI*, *BbeI*, *NotI*, *SmaI*, *XmaI*, *MboI*, *BstBI*, *ClaI*, *MluI*, *NaeI*, *NarI*, *PvuI*, *SacII*, *HhaI* and any combination thereof.~~
- ~~28. 13. The method of any one of claims 4-27 3-12, wherein the pre-amplification primer pool comprises at least one methylation-specific primer pair; preferably~~
- ~~wherein the at least one methylation-specific primer pair comprises a forward primer and a reverse primer each comprising an oligonucleotide sequence that hybridizes under stringent conditions, moderately stringent conditions, or highly stringent conditions to at least 9 consecutive nucleotides of one of the target marker(s), wherein the at least 9 consecutive nucleotides of one of the target marker(s) comprise at least one CpG site.~~
- ~~29. The method of claim 28, wherein the at least one methylation-specific primer pair comprises a forward primer and a reverse primer each comprising an oligonucleotide sequence that hybridizes under stringent conditions, moderately stringent conditions, or highly stringent conditions to at least 9 consecutive nucleotides of one of the target marker(s), wherein the at least 9 consecutive nucleotides of one of the target marker(s) comprise at least one CpG site.~~
- ~~30. The method of claim 28 or 29, wherein the pre-amplification primer pool further comprises a control primer pair for amplifying a control marker.~~
- ~~31. The method of claim 30, wherein the control marker is selected from the group consisting of ACTB, GAPDH, tubulin, ALDOA, PGK1, LDHA, RPS27A, RPL19, RPL11, ARHGDI1, RPL32, C1orf43, CHMP2A, EMC7, GPI, PSMB2, PSMB4, RAB7A, REEP5, SNRPD3, VCP, and VPS29.~~
- ~~32. The method of any one of claims 28-31, wherein the at least one methylation-specific primer pair comprises one or more pairs of nucleotide sequences selected from the group consisting of SEQ ID NOs: 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14, 15/16, 17/18, 19/20, 21/22, 23/24, 25/26, 27/28, 29/30, 31/32, 33/34, 35/36, 37/38, 39/40, 41/42, 43/44, 45/46, 47/48, 49/50, 51/52, 53/54, and 170/171.~~
- ~~33. The method of any one of claims 28-32, wherein in step (c), the at least one target marker is amplified in the presence of one or more blocker oligonucleotides.~~

- ~~34. The method of any one of the preceding claims, wherein the quantifying is conducted by polymerase chain reaction (PCR) (e.g. real time PCR, digital PCR), nucleic acid sequencing, mass-based separation (e.g. electrophoresis, mass spectrometry), or target capture (e.g. hybridization, microarray).~~
- ~~35. The method of any one of the preceding claims, wherein the quantifying is conducted by the real time PCR.~~
- ~~36. The method of claim 35, wherein the real time PCR is multiplexed real time PCR.~~
37. 14. The method of any one of claims ~~6-36~~ 4-13, wherein if step (c) is present, then the quantifying of step (d) comprises amplifying the achieved DNA from step (c) using quantification primer pair(s) and a DNA polymerase, wherein the at least a portion of the achieved DNA is amplified; if step (c) is absent, then the quantifying of step (d) comprises amplifying the at least one target marker within the treated DNA obtained from step (b) using quantification primer pair(s) and a DNA polymerase; or
- wherein if step (c) is present, then the quantification primer pair(s) used in step (d) is (are) capable of hybridizing to at least 9 consecutive nucleotides of the achieved DNA from step (c) under stringent conditions, moderately stringent conditions, or highly stringent conditions; if step (c) is absent, then the quantification primer pair(s) used in step (d) is (are) capable of hybridizing to at least 9 consecutive nucleotides of the at least one target marker within the treated DNA obtained from step (b) under stringent conditions, moderately stringent conditions, or highly stringent conditions; or
- wherein if step (c) is present, then at least one of the quantification primer pair(s) used in step (d) is (are) identical to at least one of the methylation-specific primer pair(s) in the pre-amplification primer pool of step (c); or
- wherein if step (c) is present, then the quantification primer pair(s) used in step (d) is (are) designed to amplify at least a portion within the achieved DNA from step (c); if step (c) is absent, then the quantification primer pair(s) used in step (d) is (are) designed to amplify at least a portion within the at least one target marker within the treated DNA obtained from step (b).

- ~~38. The method of claim 37, wherein if step (c) is present, then the quantification primer pair(s) used in step (d) is (are) capable of hybridizing to at least 9 consecutive nucleotides of the achieved DNA from step (c) under stringent conditions, moderately stringent conditions, or highly stringent conditions; if step (c) is absent, then the quantification primer pair(s) used in step (d) is (are) capable of hybridizing to at least 9 consecutive nucleotides of the at least one target marker within the treated DNA obtained from step (b) under stringent conditions, moderately stringent conditions, or highly stringent conditions.~~
- ~~39. The method of claim 38, wherein if step (c) is present, then at least one of the quantification primer pair(s) used in step (d) is (are) identical to at least one of the methylation-specific primer pair(s) in the pre-amplification primer pool of step (c).~~
- ~~40. The method of claim 38, wherein if step (c) is present, then the quantification primer pair(s) used in step (d) is (are) designed to amplify at least a portion within the achieved DNA from step (c); if step (c) is absent, then the quantification primer pair(s) used in step (d) is (are) designed to amplify at least a portion within the at least one target marker within the treated DNA obtained from step (b).~~
- ~~41. The method of any one of claims 35-40, wherein the step (d) is conducted in the presence of a detection agent.—~~
- ~~42. The method of claim 41, wherein the detection agent is selected from the group consisting of a fluorescent probe, an intercalating dye, a chromophore-labeled probe, a radioisotope-labeled probe, and a biotin-labeled probe.—~~
- ~~43. The method of claim 42, wherein the fluorescent probe comprises a nucleotide sequence selected from the group consisting of SEQ ID NOs: 57-85, 172.~~
- ~~44. The method of claim 42 or 43, wherein the fluorescent probe is labeled with a fluorescent dye (e.g. FAM, HEX/VIC, TAMRA, Texas Red, or Cy5) at its 5' end, and labeled with a quencher (e.g. BHQ1, BHQ2, BHQ3, DABCYL or TAMRA) at its 3' end.~~
- ~~45. 15. The method of any one of claims 35-44 4-14, wherein step (e) comprises comparing Ct value(s) of the target marker(s) of step (d) with a reference Ct value, wherein an identical or lower Ct value of at least one target marker~~

relative to its corresponding reference Ct value indicates that the subject has colorectal neoplasm, is at the onset or at a risk to the onset of colorectal neoplasm, or develops or with an increased probability of developing colorectal neoplasm, or has poor prognosis or at a risk to poor prognosis of colorectal neoplasm; or a higher Ct value of at least one target marker relative to its corresponding Ct value prior to the treatment indicates that the subject who is receiving the treatment of colorectal neoplasm is responsive to the treatment.

~~46. The method of any one of claims 4-45, wherein the pre-amplification comprises from 5 to 30 cycles of reaction, wherein each cycle comprises reaction at 85~99°C for 5s to 5 mins before reaction at 40~80°C for 5s-5mins.~~

47. 16. The method of any one of claims ~~6-46~~ 4-15, wherein if step (c) is present, then the quantifying of step (d) comprises determining the methylation level based on presence or level of a plurality of CpG dinucleotides, TpG dinucleotides, or CpA dinucleotides in the achieved DNA from step (c); if step (c) is absent, then the quantifying of step (d) comprises determining the methylation level of at least one target marker based on presence or level of a plurality of CpG dinucleotides, TpG dinucleotides, or CpA dinucleotides in the at least one target marker within the treated DNA obtained from step (b);
or

wherein if step (c) is present, then the quantifying of step (d) comprises determining methylation level of cytosine residue(s) based on presence or level of one or more CpG dinucleotides in the achieved DNA from step (c); if step (c) is absent, then the quantifying of step (d) comprises determining methylation level of cytosine residue(s) based on presence or level of one or more CpG dinucleotides in the at least one target marker within the treated DNA obtained from step (b); or

wherein if step (c) is present, then the quantifying of step (d) is performed by partitioning the achieved DNA from step (c) into a plurality of fractions; if step (c) is absent, then the quantifying of step (d) is performed by partitioning the at least one target marker within the treated DNA obtained from step (b) into a plurality of fractions.

- ~~48. The method of claim 47, wherein if step (c) is present, then the quantifying of step (d) comprises determining methylation level of cytosine residue(s) based on presence or level of one or more CpG dinucleotides in the achieved DNA from step (c); if step (c) is absent, then the quantifying of step (d) comprises determining methylation level of cytosine residue(s) based on presence or level of one or more CpG dinucleotides in the at least one target marker within the treated DNA obtained from step (b).~~
49. The method of any one of claims 6-48, wherein if step (c) is present, then the quantifying of step (d) is performed by partitioning the achieved DNA from step (c) into a plurality of fractions; if step (c) is absent, then the quantifying of step (d) is performed by partitioning the at least one target marker within the treated DNA obtained from step (b) into a plurality of fractions.—
- ~~50. The method of any one of claims 6-49, wherein the reference levels of step (e) are determined based on the clinical samples obtained from a group of individuals having or at the risk of having colorectal neoplasm and a group of individuals without or are free of the risk of having colorectal neoplasm.—~~
- ~~51.~~ 17. The method of any one of the preceding claims, wherein the colorectal neoplasm is a colorectal cancer, a large colorectal adenoma, and/or a sessile serrated polyp.
- ~~52. The method of any one of claims 1-51, wherein the colorectal neoplasm is pre-cancerous.—~~
- ~~53. The method of any one of the preceding claims, wherein the subject is a human.—~~
- ~~54.~~ 18. A kit for diagnosing colorectal neoplasm, screening for the onset or risk to the onset of colorectal neoplasm or assessing the development or prognosis of colorectal neoplasm, comprising:
- (a). a first reagent for treating a DNA, wherein the first reagent is capable of distinguishing between an unmethylated site and a methylated site in the DNA;
 - (b). optionally a first primer pool comprising at least one primer pair for pre-amplifying at least one target sequence in at least one target marker selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN,

VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5, wherein the at least one primer pair is capable of hybridizing under stringent conditions, moderately stringent conditions, or highly stringent conditions to at least 9 consecutive nucleotides of the at least one target sequence treated by the first reagent, and wherein the target sequence comprises at least one CpG site; and

- (c). a second reagent, wherein if the first primer pool is present, then the second reagent is for quantifying methylation level of the at least one target marker pre-amplified by the first primer pool; if the first primer pool is absent, then the second reagent is for quantifying methylation level of at least one target marker within the DNA treated by the first reagent, wherein the at least one target marker comprises one or more markers selected from the group consisting of Septin9, BCAT1, IKZF1, BCAN, VAV3, IRF4, POU4F2, SALL1, PKNOX2, SDC2, ASCL4, TMEFF2, SLC24A2, NDRG4, NKX2-6, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, CRHBP, INTERGENIC REGION 1, INTERGENIC REGION 2, INTERGENIC REGION 3, INTERGENIC REGION 4, and INTERGENIC REGION 5;

optionally wherein the at least one target marker comprises multiple target markers.

~~55. The kit of claim 54, wherein the at least one target marker comprises multiple target markers, wherein the multiple target markers comprise at least two markers selected from the group consisting of Septin9, BCAT1, and IKZF1.~~

~~56.~~ 19. The kit of claim ~~55~~ 18, wherein if the first primer pool is present, then the second reagent comprises a second primer pool comprising multiple quantification primer pairs capable of hybridizing under stringent conditions, moderately stringent conditions, or highly stringent conditions to at least 9

consecutive nucleotides of the at least one target sequence pre-amplified by the first primer pool; if the first primer pool is absent, then the second reagent comprises a third primer pool comprising multiple quantification primer pairs capable of hybridizing under stringent conditions, moderately stringent conditions, or highly stringent conditions to at least 9 consecutive nucleotides of the at least one target sequence of the at least one target marker within the DNA treated by the first reagent.

~~57.~~ 20. The kit of claim ~~56~~ 19, wherein at least one of the quantification primer pairs in the second primer pool is identical to at least one of the primer pairs in the first primer pool; preferably wherein if the first primer pool is present, then quantification primer pairs of the second primer pool are designed to amplify at least a portion within the at least one target sequence pre-amplified by the first primer pool; if the first primer pool is absent, then quantification primer pairs of the third primer pool are designed to amplify at least a portion within the at least one target sequence of the at least one target marker within the DNA treated by the first reagent.

~~58.~~ The kit of claim ~~56~~, wherein if the first primer pool is present, then quantification primer pairs of the second primer pool are designed to amplify at least a portion within the at least one target sequence pre-amplified by the first primer pool; if the first primer pool is absent, then quantification primer pairs of the third primer pool are designed to amplify at least a portion within the at least one target sequence of the at least one target marker within the DNA treated by the first reagent.

~~59.~~ 21. The kit of any of claims ~~54-58~~ 18-20, wherein the first, second, or third primer pool comprises at least one methylation-specific primer pair.

~~60.~~ The kit of any one of claims ~~56-59~~, wherein the first primer pool and the second primer pool are packaged in a single container or in separate containers.—

~~61.~~ The kit of any one of claims ~~55-60~~, wherein the kit further comprises one or more blocker oligonucleotides.—

~~62.~~ The kit of any one of claims ~~55-61~~, wherein the kit further comprises a detection agent.—

- ~~63. The kit of claim 62, wherein the detection agent is selected from the group consisting of a fluorescent probe, an intercalating dye, a chromophore labeled probe, a radioisotope labeled probe, and a biotin labeled probe.—~~
- ~~64. The kit of claim 63, wherein the fluorescent probe comprises an oligonucleotide sequence selected from the group consisting of SEQ ID NOs: 57-85, 172.~~
- ~~65. The kit of claim 63 or 64, wherein the fluorescent probe is labeled with a fluorescent dye (e.g. FAM, HEX/VIC, TAMRA, Texas Red, or Cy5) at its 5' end, and labeled with a quencher (e.g. BHQ1, BHQ2, BHQ3, DABCYL, TAMRA or Iowa Black Dark Quenchers) at its 3' end.~~
- ~~66. The kit of any one of claims 55-65, wherein the kit further comprises a DNA polymerase and/or a container suitable for containing the biological sample from the subject.—~~
- ~~67. The kit of any one of claims 55-66, wherein the kit further comprises an instruction for use and/or interpretation of the kit results.~~
- ~~68. The kit of any one of claims 55-67, wherein the first reagent comprises a bisulfite reagent or methylation sensitive restriction enzyme (MSRE).—~~
- ~~69. The kit of claim 68, wherein the bisulfite reagent is selected from the group consisting of ammonium bisulfite, sodium bisulfite, potassium bisulfite, calcium bisulfite, magnesium bisulfite, aluminum bisulfite, hydrogen sulfite and any combination thereof.—~~
- ~~70. The kit of claim 68, wherein the MSRE is selected from the group consisting of *HpaII*, *Sall*, *Sall* HF[®], *ScrFI*, *BbeI*, *NotI*, *SmaI*, *XmaI*, *MboI*, *BstBI*, *ClaI*, *MluI*, *NaeI*, *NarI*, *PvuI*, *SacII*, *HhaI* and any combination thereof.~~
71. 22. The kit of any one of claims ~~55-70~~ 18-21, wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of BCAN, PKNOX2, VAV3, NDRG4, and IRF4; or wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of POU4F2, SALL1, SDC2, ASCL4, INTERGENIC REGION 1, TMEFF2, INTERGENIC REGION 4, NKX2-6, INTERGENIC REGION 5, SLC24A2, INTERGENIC REGION 2,

INTERGENIC REGION 3, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, and CRHBP.

~~72. The kit of claim 71, wherein the multiple target markers further comprise one or more additional markers selected from the group consisting of POU4F2, SALL1, SDC2, ASCL4, INTERGENIC REGION 1, TMEFF2, INTERGENIC REGION 4, NKX2-6, INTERGENIC REGION 5, SLC24A2, INTERGENIC REGION 2, INTERGENIC REGION 3, KCNA6, SOX1, HS3ST2, FGF12, KCTD8, HMX1, MARCH11, and CRHBP.~~

~~73.~~ 23. The kit of any one of claims ~~54-72~~ 18-22, wherein the respective target marker comprises or is: a) the respective region defined by Hg19 coordinates as set forth below:

Target Marker	Hg19 Coordinate
NDRG4	chr16:58496750-58547532
BCAT1	chr12:24964295-25102393
IKZF1	chr7:50343720-50472799
Septin9	chr17:75276651-75496678
SDC2	chr8:97505579-97624000
VAV3	chr1:108113782-108507766
IRF4	chr6:391739-411447
TMEFF2	chr2:192813769-193060435
SALL1	chr16:51169886-51185278
BCAN	chr1:156611182-156629324
POU4F2	chr4:147560045-147563626
PKNOX2	chr11:125034583-125303285
ASCL4	chr12:108168162-108170421
KCNA6	chr12:4918342-4960277

SOX1	chr13:112721913-112726020
HS3ST2	chr16:22825498-22927659
FGF12	chr3:191857184-192485553
KCTD8	chr4:44175926-44450824
HMX1	chr4:8847802-8873543
MARCH11	chr5:16067248-16180871
CRHBP	chr5:76248538-76276983
NKX2-6	chr8:23559964-23564111
SLC24A2	chr9:19507450-19786926
INTERGENIC REGION 1	chr6:19679885-19693988
INTERGENIC REGION 2	chr10:130082033-130087148
INTERGENIC REGION 3	chr10:133107880-133113966
INTERGENIC REGION 4	chr7:152620588-152624685
INTERGENIC REGION 5	chr8:70945014-70949177

, and 5 kb upstream of the respective start site and 5 kb downstream of the respective end site of each region described above, or b) a bisulfite converted counterpart of a), or c) a MSRE treated counterpart of a).

74. ~~The kit of any one of claims 55-73, wherein if the first primer pool is present, then the first primer pool comprises at least one primer pair comprising or consisting of at least one pair of nucleotide sequences selected from the group consisting of SEQ ID NOs: 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14, 15/16, 17/18, 19/20, 21/22, 23/24, 25/26, 27/28, 29/30, 31/32, 33/34, 35/36, 37/38, 39/40, 41/42, 43/44, 45/46, 47/48, 49/50, 51/52, 53/54, 170/171, and optionally wherein the second primer pool comprises at least one primer pair that is identical to at least one of the primer pairs in the first primer pool; if the first primer pool is absent, then the third primer pool comprises at least one primer pair comprising or consisting of at least one pair of nucleotide sequences selected from the group~~

~~consisting of SEQ ID NOs: 1/2, 3/4, 5/6, 7/8, 9/10, 11/12, 13/14, 15/16, 17/18, 19/20, 21/22, 23/24, 25/26, 27/28, 29/30, 31/32, 33/34, 35/36, 37/38, 39/40, 41/42, 43/44, 45/46, 47/48, 49/50, 51/52, 53/54, and 170/171.—~~

~~75. The kit of any one of claims 56-74, wherein the first primer pool, the second primer pool, or optionally the third primer pool further comprises a primer pair for amplifying a control marker.—~~

~~76. The kit of claim 75, wherein the control marker is selected from the group consisting of ACTB, GAPDH, tubulin, ALDOA, PGK1, LDHA, RPS27A, RPL19, RPL11, ARHGDI1, RPL32, C1orf43, CHMP2A, EMC7, GPI, PSMB2, PSMB4, RAB7A, REEP5, SNRNP3, VCP, and VPS29.—~~

~~77. The kit of any one of claims 54-76, wherein the kit further comprises a plurality of containers, each for receiving a fraction of the second primer pool.—~~

~~78. Use of the kit of claims 54-77 in the manufacture of a diagnostic kit for diagnosing colorectal neoplasm, screening for the onset or risk to the onset of colorectal neoplasm, or assessing the development or prognosis of colorectal neoplasm in the subject, or monitoring treatment response in a subject who is receiving treatment of colorectal neoplasm.—~~