

CLAIMS

1. A method for detecting methylated DNA, comprising:

(a) treating a target DNA-containing sample with at
5 least one reagent which modifies a non-methylated DNA site to
be distinguished from a methylated DNA site;

(b) constructing a reverse primer, which comprises a
target-specific sequence designed to be capable of binding
complementarily to the target DNA treated with the reagent,
10 and a universal primer that does not bind complementarily to
the target DNA, and the universal primer is bound to the 5'
end of the target-specific sequence;

(c) performing one direction asymmetric linear
amplification using, as a template, the target DNA treated
15 with the reagent in step (a), and using, as a primer, the
reverse primer constructed in step (b);

(d) amplifying the target DNA using a forward primer
which is capable of binding complementarily to the DNA
linearly amplified in step (c), and a universal primer that
20 does not bind complementarily to the linearly amplified
target DNA; and

(e) detecting methylation of the target DNA by a probe
capable of hybridizing complementarily to the target DNA
sequence amplified in step (d).

2. The method of claim 1, wherein the reagent is one or more selected from among bisulfite, hydrogen sulfite, disulfite, and combinations thereof.

5 3. The method of claim 1 or 2, wherein at least one cytosine base is converted to either uracil or another base that is dissimilar to cytosine, by the treatment with the reagent.

10 4. The method of any one of claims 1 to 3, wherein a plurality of target DNA methylations are detected in the sample in a single reaction.

15 5. The method of any one of claims 1 to 4, wherein the universal primer of the step (b) or (d) comprises one or more sequences selected from the group consisting of nucleotide sequences set forth in SEQ ID NOs: 7 and 35 to 41.

20 6. The method of any one of claims 1 to 5, wherein the target-specific sequence of step (b) binds complementarily to a methylated site and/or a non-methylated site of the target DNA.

7. The method of any one of claims 1 to 6, wherein the target-specific sequence of step (b) comprises one or more CpG dinucleotides.

5 8. The method of any one of claims 1 to 7, wherein the target-specific sequence of step (b) comprises one or more sequences selected from the group of sequences set forth in SEQ ID NOs: 2, 5, 9, 14, 17, 21 and 26.

10 9. The method of any one of claims 1 to 8, wherein step (e) comprises detecting methylation of the target DNA using one or more methods selected from the group consisting of PCR, methylation specific PCR, real-time methylation specific PCR, PCR using a methylated DNA-specific binding protein, PCR
15 using a methylated DNA-specific binding antibody, quantitative PCR, DNA chip Assay, sequencing, sequencing-by-synthesis, and sequencing-by-ligation.

20 10. The method of any one of claims 1 to 9, wherein the forward primer of step (d) comprises one or more CpG dinucleotides.

 11. The method of any one of claims 1 to 9, wherein the forward primer of step (d) comprises one or more sequences

selected from the group of sequences set forth in SEQ ID NOs:
1, 4, 8, 10 to 13, 15, 16, 18 to 20, 22 to 25, 27 and 28.

12. The method of any one of claims 1 to 11, wherein the
5 probe of step (e) comprises one or more CpG dinucleotides.

13. The method of any one of claims 1 to 12, wherein the
probe of step (e) comprises one or more sequences selected
from the group of sequences set forth in SEQ ID NOs: 3, 6, 29,
10 30, and 31 to 34.

14. The method of any one of claims 1 to 13, wherein the
probe of step (e) further comprises a reporter or quencher.

15 15. The method of any one of claims 1 to 14, wherein the
forward primer of the step (d) and the universal primer of
the step (d) have a self-reporting function or are energy
transfer-labeled.

20 16. The method of claim 14, wherein the reporter is
selected from the group consisting of FAM (6-
carboxyfluorescein), Texas red, HEX (2', 4', 5', 7',-
tetrachloro-6-carboxy-4,7-dichlorofluorescein), JOE, Cy3, and
Cy5.

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17. The method of claim 14, wherein the quencher is selected from the group consisting of TAMRA (6-carboxytetramethyl-rhodamine), BHQ1, BHQ2 and Dabcyl.

5 18. The method of any one of claims 1 to 17, wherein the target DNA is SDC2.

19. A composition for detecting methylated DNA, comprising:

10 a reverse primer which comprises a target-specific sequence capable of binding complementarily to a target DNA sequence treated with a reagent which modifies a non-methylated DNA to be distinguished from a methylated DNA, and a universal primer that does not bind complementarily to the
15 target DNA, fused to the 5' end of the target-specific sequence;

a forward primer capable of binding complementarily to a linearly amplified target DNA, and a universal primer not complementary to the linearly amplified target DNA; and

20 a probe capable of hybridizing complementarily to the linearly amplified target DNA sequence.

20. The composition of claim 19, wherein the reagent is selected from the group consisting of bisulfite, hydrogen
25 sulfite, disulfite, and combinations thereof.

21. The composition of claim 19 or 20, wherein the composition is formulated to detect methylation of a plurality of target DNAs in a sample in a single reaction.

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22. The composition of any one of claims 19 to 21, wherein the universal primer comprises one or more sequences selected from the group of nucleotide sequences set forth in SEQ ID NOs: 7 and 35 to 41.

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23. The composition of any one of claims 19 to 22, wherein the target-specific sequence binds complementarily to a methylated site and/or a non-methylated site of the target DNA.

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24. The composition of any one of claims 19 to 23, wherein the target-specific sequence comprises one or more CpG dinucleotides.

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25. The composition of any one of claims 19 to 24, wherein the target-specific sequence comprises one or more sequences selected from the group of sequences set forth in SEQ ID NOs: 2, 5, 9, 14, 17, 21 and 26.

26. The composition of any one of claims 19 to 25, wherein the forward primer comprises one or more CpG dinucleotides.

5 27. The composition of any one of claims 19 to 26, wherein the forward primer comprises one or more sequences selected from the group of sequences set forth in SEQ ID NOs: 1, 4, 8, 10 to 13, 15, 16, 18 to 20, 22 to 25, 27 and 28.

10 28. The composition of any one of claims 19 to 27, wherein the probe comprises one or more CpG dinucleotides.

 29. The composition of any one of claims 19 to 28, wherein the probe comprises one or more sequences selected
15 from the group of sequences set forth in SEQ ID NOs: 3, 6, 29, 30, and 31 to 34.

 30. A kit for detecting methylated DNA, which comprises the composition of any one of claims 19 to 29.

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