

Claims:

1. A method for prenatal diagnosis of a fetal aneuploidy using a sample of maternal blood, the method comprising:

5 a) in a sample of maternal blood, physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypomethylated DNA or hypermethylated DNA, to obtain a hypermethylated DNA sample;

b) in the hypermethylated DNA sample, determining levels of a plurality of differentially methylated regions (DMRs) to obtain a hypermethylation value for the
10 hypermethylated DNA sample;

c) comparing the hypermethylation value of the hypermethylated DNA sample to a standardized reference hypermethylation value for said plurality of DMRs; and

d) diagnosing a fetal aneuploidy based on said comparison.

15 2. The method of claim 1, wherein the maternal blood sample is a maternal peripheral blood sample.

3. The method of claim 1 or 2, wherein hypermethylated DNA is separated from hypomethylated DNA using an antibody that immunoprecipitates methylated DNA.

20 4. The method of any one of the preceding claims, wherein the plurality of DMRs are chosen from the lists shown in Appendices A-E.

5. The method of any one of the preceding claims, wherein the levels of the plurality of
25 DMRs is determined for at least three, five, eight, or ten DMRs.

6. The method of any one of the preceding claims, wherein the levels of the plurality of DMRs in the hypermethylated DNA sample are determined by real time quantitative polymerase chain reaction (Real Time QPCR).

7. The method of any one of the preceding claims, wherein the hypermethylation value for the hypermethylated DNA sample is compared to a standardized normal reference hypermethylation value for a normal maternal blood DNA sample and diagnosis of fetal aneuploidy is made when the hypermethylation value for the hypermethylated DNA sample is higher than the standardized normal reference hypermethylation value for a normal maternal blood DNA sample.

8. The method of any one of the preceding claims, wherein the plurality of DMRs are on chromosome 21 for diagnosis of trisomy 21.

9. The method of any one of the preceding claims, wherein the plurality of DMRs are on chromosome 21 and:

(a) comprise three or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof;

(b) comprise eight or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof; or

(c) consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs

22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).

5 10. The method of any one of claims 1-7, wherein the plurality of DMRs are on a chromosome selected from the group consisting of chromosome 21, chromosome 13, chromosome 18, X chromosome and Y chromosome.

10 11. A method for prenatal diagnosis of trisomy 21 using a sample of maternal peripheral blood, the method comprising:

a) in a sample of maternal peripheral blood, physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypermethylated DNA or hypomethylated DNA, to obtain a hypermethylated DNA sample;

15 b) in the hypermethylated DNA sample, determining levels of a plurality of differentially methylated regions (DMRs) on chromosome 21 to obtain a hypermethylation value for the hypermethylated DNA sample,

wherein the plurality of DMRs on chromosome 21 comprise eight or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33),
20 base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42),
25 base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof, and

wherein the levels of the plurality of DMRs are determined by real time quantitative polymerase chain reaction (Real Time QPCR);

30 c) comparing the hypermethylation value of the hypermethylated DNA sample to a standardized normal reference hypermethylation value for said plurality of DMRs on chromosome 21; and

d) diagnosing trisomy 21 based on said comparison.

12. The method of any one of the preceding claims, wherein hypermethylated DNA is physically separated from hypomethylated DNA by Methylation DNA Immunoprecipitation (MeDiP).

13. The method of any one of the preceding claims, wherein, after physical separation of hypermethylated DNA from hypomethylated DNA, the hypermethylated DNA is amplified.

14. The method of any one of the preceding claims, wherein the hypermethylated DNA is amplified by ligation-mediated polymerase chain reaction (LM-PCR).

15. The method of any one of the preceding claims, wherein the levels of the plurality of DMRs are also determined in a total untreated maternal blood DNA sample as a control of the LM-PCR efficiency.

16. The method of claim 11, wherein the hypermethylation value for the hypermethylated DNA sample is compared to a standardized normal reference hypermethylation value for a normal maternal blood DNA sample, and diagnosis of trisomy 21 is made when the hypermethylation value for the hypermethylated DNA sample is higher than the standardized normal reference hypermethylation value for a normal maternal blood DNA sample.

17. The method of claim 11, wherein the plurality of DMRs on chromosome 21 consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).

18. A method for identifying a differentially methylated region (DMR) on a chromosome of interest suitable for use in diagnosing a fetal aneuploidy, the method comprising:

a) providing:

(i) a normal adult female peripheral blood DNA sample (PB sample); and

(ii) a normal placental DNA sample (PL sample);

b) in each sample of a), physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypermethylated DNA or hypomethylated DNA, to obtain:

(i) a separated PB sample; and

(iii) a separated PL sample;

c) in each separated sample of b), determining levels of a plurality of regions on a chromosome of interest; and

d) selecting a region that is hypomethylated in the separated PB sample and is hypermethylated in the separated PL sample to thereby identify a differentially methylated region (DMR) on the chromosome of interest.

19. The method of claim 18, wherein the PL sample comprises two different samples, a first trimester PL sample and a third trimester PL sample, wherein step d) further comprises selecting a region having an equivalent degree of methylation in the first trimester separated PL sample and the third trimester separated PL sample.

20. The method of claim 18 or 19, wherein the chromosome of interest is selected from the group consisting of chromosome 21, chromosome 13, chromosome 18, X chromosome and Y chromosome.

21. The method of claim 18 or 19, wherein the aneuploidy is a trisomy or a monosomy.

22. A kit for prenatal diagnosis of trisomy 21, the kit comprising:

a) one or more nucleic acid compositions for determining levels of a plurality of differentially methylated regions (DMRs) on chromosome 21; and

b) instructions for using the nucleic acid compositions for prenatal diagnosis of trisomy 21.

23. The kit of claim 22, wherein the plurality of DMRs on chromosome 21 are chosen from the lists shown in Appendices A-E.

24. The kit of claim 22, wherein the plurality of DMRs on chromosome 21:

(a) comprise three or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof;

(b) comprise eight or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof ; or

(c) consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).

25. The kit of any one of claims 22-24, wherein the nucleic acid compositions comprise one or more oligonucleotide primers selected from the group consisting of GCTGGACCAGAAAGTGTTGAG (SEQ ID NO: 1), GTGTGCTGCTTTGCAATGTG (SEQ ID NO: 2), GGTCGAGTTTTTGGTGGTGT (SEQ ID NO: 3),
5 CCACCGTCACTGTTCTAGTA (SEQ ID NO: 4), CCTCGTGCTCGTGTCTGTAT (SEQ ID NO: 5), GAGGAAACAGCTTGGCTCTG (SEQ ID NO: 6), CTGTTGCATGAGAGCAGAGG (SEQ ID NO: 7), CGTCCCCCTCGCTACTATCT (SEQ ID NO: 8), TGCAGGATATTTGGCAAGGT (SEQ ID NO: 9), CTGTGCCGGTAGAAATGGTT (SEQ ID NO: 10), TGAATCAGTTCACCGACAGC
10 (SEQ ID NO: 11), GAAACAACCTGGCCATTCTC (SEQ ID NO: 12), CCGTTATATGGATGCCTTGG (SEQ ID NO: 13), AACTGTTGGGCTGAACTGC (SEQ ID NO: 14), CCAGGCAAGATGGCTTATGT (SEQ ID NO: 15), ACCATGCTCAGCCAATTTTT (SEQ ID NO: 16), GACCCAGACGATACCTGGAA (SEQ ID NO: 17), GCTGAACAAAACCTCGGCTTC (SEQ ID NO: 18),
15 CCACATCCTGGCCATCTACT (SEQ ID NO: 19), TTCCACAGACAGCAGAGACG (SEQ ID NO: 20), TGAGCTCACAGGTCTGGAAA (SEQ ID NO: 21), CCCACAGGGTTCTGGTAAT (SEQ ID NO: 22), ATTCTCCACAGGGCAATGAG (SEQ ID NO: 23), TTATGTGGCCTTTCCTCCTG (SEQ ID NO: 24), and combinations thereof.

20 26. The kit of any one of claims 22-25, which further comprises means for physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypermethylated DNA or hypomethylated DNA, in a blood sample.

25 27. The kit of claim 26, wherein the means for physically separating hypermethylated DNA from hypomethylated DNA comprises an antibody that immunoprecipitates methylated DNA.

30 28. The kit of claim 26, which further comprises means for amplifying hypermethylated DNA.

29. The kit of claim 28, wherein the means for amplifying hypermethylated DNA comprises oligonucleotide linkers or oligonucleotide primers for performing ligation mediated polymerase chain reaction (LM-PCR).

- 5 30. A nucleic acid composition comprising one or more isolated oligonucleotide primers selected from the group consisting of GCTGGACCAGAAAGTGTGAG (SEQ ID NO: 1), GTGTGCTGCTTTGCAATGTG (SEQ ID NO: 2), GGTCGAGTTTTGGTGGTGT (SEQ ID NO: 3), CCACCGTCACTGTTCTCTAGA (SEQ ID NO: 4), CCTCGTGCTCGTGTCTGTAT (SEQ ID NO: 5), GAGGAAACAGCTTGGCTCTG (SEQ ID NO: 6), CTGTTGCATGAGAGCAGAGG (SEQ ID NO: 7),
10 CGTCCCCCTCGCTACTATCT (SEQ ID NO: 8), TGCAGGATATTTGGCAAGGT (SEQ ID NO: 9), CTGTGCCGGTAGAAATGGTT (SEQ ID NO: 10), TGAATCAGTTCACCGACAGC (SEQ ID NO: 11), GAAACAACCTGGCCATTCTC (SEQ ID NO: 12), CCGTTATATGGATGCCTTGG (SEQ ID NO: 13),
15 AAAGTGTGGGCTGAACTGC (SEQ ID NO: 14), CCAGGCAAGATGGCTTATGT (SEQ ID NO: 15), ACCATGCTCAGCCAATTTT (SEQ ID NO: 16), GACCCAGACGATACCTGGAA (SEQ ID NO: 17), GCTGAACAAAACCTCGGCTTC (SEQ ID NO: 18), CCACATCCTGGCCATCTACT (SEQ ID NO: 19), TTCCACAGACAGCAGAGACG (SEQ ID NO: 20), TGAGCTCACAGGTCTGGAAA (SEQ ID NO: 21), CCCACAGGGTTCTGGTAAT (SEQ ID NO: 22),
20 ATTCTCCACAGGGCAATGAG (SEQ ID NO: 23), TTATGTGGCCTTTCCTCCTG (SEQ ID NO: 24), and combinations thereof.

CLAIMS

1. A method for prenatal diagnosis of trisomy 21 using a sample of maternal blood, the method comprising:
 - 5 a) in a sample of maternal blood, physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypomethylated DNA or hypermethylated DNA, to obtain a hypermethylated DNA sample;
 - b) in the hypermethylated DNA sample, determining levels of a plurality of
10 differentially methylated regions (DMRs) to obtain a hypermethylation value for the hypermethylated DNA sample, wherein at least one of the DMRs comprises a region selected from base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557- 42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID
15 NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 32268843-32268943 (SEQ ID NO: 42) and base pairs 37841284-37841411 (SEQ ID NO: 44);
 - c) comparing the hypermethylation value of the hypermethylated DNA sample to a standardized reference hypermethylation value for said plurality of DMRs; and
 - d) diagnosing trisomy 21 based on said comparison.
- 20 2. The method of claim 1, wherein the maternal blood sample is a maternal peripheral blood sample or a fractionated portion of maternal peripheral blood.
3. The method of claim 1 or claim 2, wherein hypermethylated DNA is separated
25 from hypomethylated DNA using an antibody that immunoprecipitates methylated DNA.
4. The method of any one of the preceding claims, wherein the plurality of DMRs are chosen from the list shown in Appendix A.
- 30 5. The method of any one of the preceding claims, wherein the levels of the plurality of DMRs is determined for at least three, five, eight, or ten DMRs.

6. The method of any one of the preceding claims, wherein the levels of the plurality of DMRs in the hypermethylated DNA sample are determined by real time quantitative polymerase chain reaction (Real Time QPCR).
- 5 7. The method of any one of the preceding claims, wherein the hypermethylation value for the hypermethylated DNA sample is compared to a standardized normal reference hypermethylation value for a normal maternal blood DNA sample and diagnosis of fetal aneuploidy is made when the hypermethylation value for the hypermethylated DNA sample is higher than the standardized normal reference
10 hypermethylation value for a normal maternal blood DNA sample.
8. The method of any one of the preceding claims, wherein the plurality of DMRs are on chromosome 21 and:
 - (a) comprise three or more regions selected from the group consisting of base
15 pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-
20 29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof;
 - (b) comprise eight or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID
25 NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base
30 pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof; or
 - (c) consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341

(SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).

- 5 9. A method for prenatal diagnosis of trisomy 21 using a sample of maternal peripheral blood, the method comprising:
- a) in a sample of maternal peripheral blood, physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypermethylated DNA or hypomethylated DNA, to obtain a
- 10 hypermethylated DNA sample;
- b) in the hypermethylated DNA sample, determining levels of a plurality of differentially methylated regions (DMRs) on chromosome 21 to obtain a hypermethylation value for the hypermethylated DNA sample,
- wherein the plurality of DMRs on chromosome 21 comprise eight or more
- 15 regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 32268843-32268943 (SEQ ID NO: 42) and base pairs 37841284-
- 20 37841411 (SEQ ID NO: 44), and combinations thereof, and
- wherein the levels of the plurality of DMRs are determined by real time quantitative polymerase chain reaction (Real Time QPCR);
- c) comparing the hypermethylation value of the hypermethylated DNA sample to a standardized normal reference hypermethylation value for said plurality of DMRs on
- 25 chromosome 21; and
- d) diagnosing trisomy 21 based on said comparison.

10. The method of any one of the preceding claims, wherein hypermethylated DNA is physically separated from hypomethylated DNA by Methylation DNA
- 30 Immunoprecipitation (MeDiP).

11. The method of any one of the preceding claims, wherein, after physical separation of hypermethylated DNA from hypomethylated DNA, the hypermethylated DNA is amplified.

12. The method of any one of the preceding claims, wherein the hypermethylated DNA is amplified by ligation-mediated polymerase chain reaction (LM-PCR).
- 5 13. The method of any one of the preceding claims, wherein the levels of the plurality of DMRs are also determined in a total untreated maternal blood DNA sample as a control of the LM-PCR efficiency.
14. The method of claim 9, wherein the hypermethylation value for the
10 hypermethylated DNA sample is compared to a standardized normal reference hypermethylation value for a normal maternal blood DNA sample, and diagnosis of trisomy 21 is made when the hypermethylation value for the hypermethylated DNA sample is higher than the standardized normal reference hypermethylation value for a normal maternal blood DNA sample.
- 15 15. The method of claim 9, wherein the plurality of DMRs on chromosome 21 consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO:
20 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).
16. A kit when used in a method of any one of the preceding claims, the kit
25 comprising:
- a) two or more nucleic acid compositions for determining levels of a plurality of differentially methylated regions (DMRs) on chromosome 21, wherein at least one of the DMRs comprises a region selected from base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 33320735-33320829
30 (SEQ ID NO: 36), base pairs 42189557- 42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 32268843-32268943 (SEQ ID NO: 42) and base pairs 37841284-37841411 (SEQ ID NO: 44); and

b) instructions for using the nucleic acid compositions for prenatal diagnosis of trisomy 21.

17. The kit of claim 16, wherein the plurality of DMRs on chromosome 21 are
5 chosen from the list shown in Appendix A.

18. The kit of claim 16, wherein the plurality of DMRs on chromosome 21:

- (a) comprise three or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO:
10 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs
15 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO: 44), and combinations thereof;
- (b) comprise eight or more regions selected from the group consisting of base pairs 39279856-39280004 (SEQ ID NO: 33), base pairs 44161178-44161323 (SEQ ID NO: 34), base pairs 44161239-44161371 (SEQ ID NO: 35), base pairs 33320735-33320829
20 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 29136735-29136844 (SEQ ID NO: 41), base pairs 32268843-32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43), base pairs 37841284-37841411 (SEQ ID NO:
25 44), and combinations thereof; or
- (c) consist of eight regions having nucleotide sequences of base pairs 33320735-33320829 (SEQ ID NO: 36), base pairs 42189557-42189683 (SEQ ID NO: 37), base pairs 42355712-42355815 (SEQ ID NO: 38), base pairs 42357215-42357341 (SEQ ID NO: 39), base pairs 22403649-22403792 (SEQ ID NO: 40), base pairs 32268843-
30 32268943 (SEQ ID NO: 42), base pairs 44079235-44079322 (SEQ ID NO: 43) and base pairs 37841284-37841411 (SEQ ID NO: 44).

19. The kit of any one of claims 16-18, wherein the nucleic acid compositions comprise one or more oligonucleotide primer pairs selected from the group consisting

- of: GCTGGACCAGAAAGTGTTGAG (SEQ ID NO: 1) and
GTGTGCTGCTTTGCAATGTG (SEQ ID NO: 2); GGTCGAGTTTTTGGTGGTGT
(SEQ ID NO: 3) and CCACCGTCACTGTTCTCTAGA (SEQ ID NO: 4);
CCTCGTGCTCGTGTCTGTAT (SEQ ID NO: 5) and
5 GAGGAAACAGCTTGGCTCTG (SEQ ID NO: 6); CTGTTGCATGAGAGCAGAGG
(SEQ ID NO: 7) and CGTCCCCCTCGCTACTATCT (SEQ ID NO: 8);
TGCAGGATATTTGGCAAGGT (SEQ ID NO: 9) and
CTGTGCCGGTAGAAATGGTT (SEQ ID NO: 10); TGAATCAGTTCACCGACAGC
(SEQ ID NO: 11) and GAAACAACCTGGCCATTCTC (SEQ ID NO: 12);
10 CCGTTATATGGATGCCTTGG (SEQ ID NO: 13) and
AAACTGTTGGGCTGAACTGC (SEQ ID NO: 14); CCAGGCAAGATGGCTTATGT
(SEQ ID NO: 15) and ACCATGCTCAGCCAATTTTT (SEQ ID NO: 16);
GACCCAGACGATACCTGGAA (SEQ ID NO: 17) and
GCTGAACAAAACCTCGGCTTC (SEQ ID NO: 18); CCACATCCTGGCCATCTACT
15 (SEQ ID NO: 19) and TTCCACAGACAGCAGAGACG (SEQ ID NO: 20);
TGAGCTCACAGGTCTGGAAA (SEQ ID NO: 21) and
CCCCACAGGGTTCTGGTAAT (SEQ ID NO: 22); ATTCTCCACAGGGCAATGAG
(SEQ ID NO: 23) and TTATGTGGCCTTTCCTCCTG (SEQ ID NO: 24); and
combinations thereof.
- 20
20. The kit of any one of claims 16-19, which further comprises means for physically separating hypermethylated DNA from hypomethylated DNA, without chemically altering or enzymatically digesting the hypermethylated DNA or hypomethylated DNA, in a blood sample.
- 25
21. The kit of claim 20, wherein the means for physically separating hypermethylated DNA from hypomethylated DNA comprises an antibody that immunoprecipitates methylated DNA.
- 30
22. The kit of claim 20, which further comprises means for amplifying hypermethylated DNA.

23. The kit of claim 22, wherein the means for amplifying hypermethylated DNA comprises oligonucleotide linkers or oligonucleotide primers for performing ligation mediated polymerase chain reaction (LM-PCR).