

CLAIMS

1. **A method** for detecting in a sample from an individual an amount of a species of DNA originating from cells of a given type, which sample comprises said species of DNA in admixture with differently methylated DNA not originating from cells of said type; said method comprising the steps:
- 5 (a) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA;
- (b) detecting in said sample the presence of methylation in said species of DNA at one or more differentially methylated region(s) (DMR(s)) that is(are) differently methylated between said species of DNA and the DNA not originating from cells of said type, the modification of DNA of such DMR by said reagent is sensitive to methylation of DNA, wherein the presence of methylated DNA at said DMR indicates the presence of said amount of species of DNA in said sample and the absence of methylated DNA at said DMR indicates the absence of said species of DNA in said sample; and
- 10 (c) detecting an amount of total DNA present in said sample using at least one other region that is not differently methylated between said species of DNA and the DNA not originating from cells of said type, the modification of which region(s) by said reagent is insensitive to methylation of DNA, wherein, said other region is located between about 20bp and about 20kb upstream or downstream of said DMR.
2. The method of claim 1, wherein said detection in step (b) and said detection in step (c) are made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously for such DMR and other region(s), and using: (x) a detectable label(s) for said DMR(s); and (y) a different detectable label(s) for said other region(s).
3. The method of claim 1 or 2, wherein said detection in step (b) comprises using two or more of said DMRs.
4. The method of claim 3, wherein: (x) the same detectable label(s) is(are) used for each of said DMRs; or (y) a different detectable label(s) is(are) used for each of said DMRs.
5. The method of any one of claims 1 to 4, wherein prior to or as part of said detection in step (b) and/or step (c), each DNA region comprising said DMR(s) and/or said other region(s), respectively, is(are) amplified.
6. The method of any one of claims 1 to 5, wherein each detectable label used in step (b) and/or step (c), is independently selected from the group consisting of: fluorescent, protein, small molecule or radioactive label.
7. The method of any one of claims 1 to 6, wherein said detection in step (b) comprises real-time quantitative probe-based PCR using at least one labelled probe specific for one of said DMR(s).
8. The method of any one of claims 1 to 7, wherein said detection in step (c) comprises real-time quantitative probe-based PCR using at least one labelled probe specific for one of said other region(s).
9. The method of any one of claims 1 to 8, wherein said other region is located upstream or downstream of said DMR within a distance selected from the group consisting of: between about 16kb to 20bp, 14kb to 20bp, 12kb to 20bp, 10kb to 20bp, 8kb to 20bp, 6kb to 20bp, 5kb to 20bp, 4kb to 20bp, 3kb to 20bp, 16kb to 20bp, 1kb to

20bp, 500bp to 20bp, 200bp to 20bp, 20kb to 15kb, 15kb to 10kb, 12kb to 8kb, 10kb to 8kb, 11kb to 7kb, 11kb to 10kb, 9kb to 8kb, 8kb to 6kb, 6kb to 4kb, 4kb to 2kb, 2kb to 500bp, 1kb to 100bp, 500bp to 50bp, 400bp to 200bp and 500bp to 100bp.

- 5 10. The method of any one of claims 1 to 9, wherein said detection in step (c) comprises using at least two of said other regions; *preferably wherein*, the number of said other regions is the same as the number of DMRs used in step (b); *more preferably wherein*, one of said other regions is located between about 20bp and about 20kb upstream or downstream of a DMR used in step (b) and each other of the said other regions is located between about 20bp and about 20kb upstream or downstream of another of said DMRs.
- 10 11. The method of claim 10, wherein said detection in step (c) is made using: (x) the same detectable label(s) for each of said other regions; or (y) a different detectable label(s) for each of said other regions.
- 15 12. The method of claim 10 or 11, wherein said detection in step (c) comprises multiplex real-time probe-based quantitative PCR using at least two labelled probes each of which is specific for one of said other regions.
- 20 13. The method of any one of claims 1 to 12, wherein said detection in step (c) and said detection in step (b) are made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously with each other, and by multiplex real-time quantitative probe-based PCR using at least one labelled probe specific for each of said DMR(s) and other regions(s).
- 25 14. The method any one of claims 1 to 13, wherein said species of DNA originates from cells of a foetus and/or the placenta of a foetus and said sample is from a pregnant female; *preferably wherein*, said species of DNA is circulating cell-free DNA and said sample is a blood fraction such as plasma or serum.
- 30 15. The method of claim 14, wherein said DMR(s) comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and at least one of said DMR(s) is located in a portion of the genome and/or gene selected from the group consisting of: RASSF1A, TBX3, HLCS, ZFY, CDC42EP1, MGC15523, SOX14 and SPN; *preferably wherein*,
- 35 • two or more of said DMRs are used in detection step (b) and each of said DMRs is located in a portion of the genome and/or gene selected from the group consisting of: RASSF1A, TBX3, HLCS, ZFY, CDC42EP1, MGC15523, SOX14 and SPN; *and/or*
- said DMR is located between about positions 4,700bp and 5,600bp of RASSF1A *or* about positions 1,660bp and 2,400bp of TBX3; *more preferably wherein*,
- 40 • two or more of said DMRs are used in detection step (b) and comprise those located between about positions 4,700bp and 5,600bp of RASSF1A *and* about positions 1,660bp and 2,400bp of TBX3.
16. The method of claim 14 or 15, wherein said other region is located in a portion of the genome and/or gene selected from the group consisting of: RASSF1A, TBX3, HLCS, ZFY, CDC42EP1, MGC15523, SOX14 and SPN; *preferably wherein*,
- said other region comprises a region without a methylation site specific for said reagent and said locus is located in the genes RASSF1A *or* TBX3, *more preferably wherein*,

- two or more of said other regions are used in detection step (c) and comprise those located between about positions 14,220bp and 13,350bp of RASSF1A and about positions 12,400bp and 13,000bp of TBX3.

- 5 17. The method any one of claims 14 to 16, wherein said pregnant female is susceptible to a pregnancy-associated medical condition; *preferably wherein*, said pregnancy-associated medical condition is selected from the group consisting of: preeclampsia, preterm labour, intrauterine growth retardation and vanishing twin.
- 10 18. The method of any one of claims 1 to 13, wherein said species of DNA originates from a cell type associated with a medical condition; *preferably wherein*, said medical condition is one selected from the group consisting of: a cell proliferative disorder, an infection/infectious disease, a wasting disorder, a degenerative disorder, an (auto)immune disorder, kidney disease, liver disease, inflammatory disease, acute toxicity, chronic toxicity, myocardial infarction, and a combination of any of the foregoing; *more preferably wherein*, said species of DNA is circulating cell-free DNA and said sample is a blood fraction such as plasma or serum.
- 15 19. The method of claim 18, wherein said species of DNA originates from cells of a tumour; *preferably wherein*, said tumour is a carcinoma or cancer of an organ selected from the group consisting of: liver, lung, breast, colon, oesophagus, prostate, ovary, cervix, uterus, testis, brain, bone marrow and blood.
- 20 20. The method of claim 19, wherein said DMR(s) comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and at least one of said DMR(s) is located in a portion of the genome and/or a gene selected from the group consisting of: a tumour suppressor gene, p16, SEPT9, RASSF1A, GSTP1, DAPK, ESR1, APC, HSD17B4 and H1C1; *preferably wherein*, said DMR is located in RASSF1A; *more preferably wherein*, said DMR is located between about positions 4,700bp and 5,600bp of RASSF1A; and/or more preferably
- 25 *wherein*, said other region is located between about positions 14,220bp and 13,350bp of RASSF1A.
- 30 21. The method of any one of claims 1 to 20, wherein said sample is a tissue sample or a sample of biological fluid; *preferably wherein*, said sample is a sample of biological fluid selected from the group consisting of: whole blood, a blood fraction, urine, saliva, sweat, ejaculate, tears, phlegm, vaginal secretion, vaginal wash and colonic wash; *more preferably wherein*, said sample is a plasma or serum sample.
22. The method of any one of claims 1 to 21, wherein said reagent that differentially modifies methylated and non-methylated DNA comprises bisulphite.
- 35 23. The method of any one of claims 1 to 21, wherein said reagent that differentially modifies methylated and non-methylated DNA comprises an agent that selectively digests unmethylated DNA over methylated DNA, *preferably wherein*, said agent comprises:
- at least one methylation sensitive enzyme;
 - at least one methylation sensitive restriction enzyme; and/or
 - an agent selected from the group consisting of: AatII, AclI, AclI, AfeI, AgeI, AgeI-HF, AscI, AsiSI, AvaI, BceAI, BmgBI, BsaAI, BsaHI, BsiEI, BsiWI, BsmBI, BspDI, BsrFI, BssHII, BstBI, BstXI, ClaI, EagI, FauI, FseI, FspI, HaeII, HgaI, HhaI, HinfII, HpaII, Hpy99I, HpyCH4IV, KasI, MluI, NaeI, NarI, NgoMIV, NotI, NotI-HF, NruI, Nt.BsmAI, Nt.CviPII, PaeR7I, PluTI, PmlI, PvuI, PvuI-HF, RsrII, SacII, SalI, SalI-HF, SfoI, SgrAI, SmaI, SnaBI, TspMI and ZraI.
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24. The method of any one of claims 1 to 23, wherein each of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as a relative concentration of said species of DNA to the total DNA in said sample.

25. The method of any one of claims 1 to 23, further comprising the steps:

- detecting an amount of total DNA in a standard sample of DNA of known amount using the same other region(s) as used in step (c); and
- comparing the signal detected from said standard sample of DNA to the signal detected in step (c).

26. The method of claim 25, wherein each of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as an absolute amount of said species of DNA in said sample.

27. The method of claim 24 or 26, further comprising the step:

- comparing the amount of said species of DNA detected with a threshold amount and/or reference distribution of amounts, wherein: (x) an increase in, or outlying of, the amount of said species of DNA indicates an increased risk of the individual suffering from or developing a medical condition; and/or (y) an amount of said species of DNA in excess to said threshold, or outlying from said distribution, indicates that a diagnosis for an abnormality in the said species of DNA present in said sample may be performed on, preferably a separate aliquot of DNA of, said sample.

28. The method of any one of claims 24 to 27, further comprising the step:

- performing on, preferably with a separate aliquot of DNA of, said sample, a diagnosis for an abnormality in said species of DNA present in said sample; *preferably wherein*, said species of DNA originates from cells of a foetus and/or the placenta of a foetus, said sample is from a pregnant female and said diagnosis is a prenatal diagnosis.

29. The method of claim 28, wherein said diagnosis comprises a step that uses a detection technology selected from the group consisting of: DNA sequencing, SNP analysis, digital PCR and hybridisation; *preferably wherein*, said detection technology is massively parallel sequencing of DNA; *more preferably wherein* said detection technology is massively parallel sequencing of random and/or enriched DNA.

30. The method of claim 28 or 29, wherein:

- (x) said species of DNA originates from cells of a foetus and/or the placenta of a foetus, said sample is from a pregnant female and said abnormality is a genetic mutation or a chromosomal abnormality, such as a chromosomal trisomy, associated with a foetal abnormality and/or a congenital disorder; *preferably wherein*:
- said genetic mutation is selected from the group consisting of: colour blindness, cystic fibrosis, hemochromatosis, haemophilia, phenylketonuria, polycystic kidney disease, sickle-cell and disease, Tay-Sachs disease; and/or
 - said chromosomal abnormality is selected from the group consisting of: a trisomy (such as trisomy 21, trisomy 18, or trisomy 13), a sex-chromosome abnormality (such as Turners syndrome, Klinefelter syndrome, [Noonan syndrome,] Triple X syndrome, XXY syndrome, or Fragile X syndrome or XYY syndrome or XXYY syndrome), a chromosomal deletion (such as Prader-Willi syndrome, Cris-du-chat syndrome, Wolf-

Hirschhorn syndrome, or 22q11 deletion syndrome, Duchene muscular dystrophy), Beckwith-Wiedemann syndrome, Canvan syndrome, and neurofibromatosis; or

(y) said species of DNA originates from cells of a tumour and said abnormality is a genetic mutation or a chromosomal abnormality associated with the diagnosis, prognosis or predictive treatment of a carcinoma or cancer; *preferably wherein*:

- said genetic mutation is selected from the group consisting of: a mutation in a tumour suppressor gene (such as TP53 (p53), BRCA1, BRCA2, APC or RB1), a mutation in a proto-oncogene (such as RAS, WNT, MYC, ERK, or TRK) and a DNA repair gene (such as HMG1, HMG2, MGMT or PMS2); and/or
- said chromosomal abnormality is selected a translocation (such as t(9;22)(q34;q11) [ie, Philadelphia chromosome or BCL-ABL], t(8;14)(q24;q32), t(11;14)(q13;q32), t(14;18)(q32;q21), t(10;(various))(q11;(various)), t(2;3)(q13;p25), t(8;21)(q22;q22), t(15;17)(q22;q21), t(12;15)(p13;q25), t(9;12)(p24;p13), t(12;21)(p12;q22), t(11;18)(q21;q21), t(2;5)(p23;q35), t(11;22)(q24;q11.2-12), t(17;22), t(1;12)(q21;p13), t(X;18)(p11.2;q11.2), t(1;19)(q10;p10), t(7;16)(q32-34;p11), t(11;16)(p11;p11), t(8;22)(q24;q11) or t(2;8)(p11;q24)).

31. The method of claim 14, wherein said DMR(s) is/are hypermethylated in foetal DNA and hypo methylated in maternal DNA.

32. The method claim 31, wherein said DMR(s) comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and at least one of said DMR(s) is located in a region and/or gene selected from the list(s) consisting of one disclosed in WO 2011/034631 as being hypermethylated in foetal DNA relative to maternal DNA, including SEQ ID NOs 1-59, 90-163, 176, 179, 180, 184, 188, 189, 190, 191, 193, 195, 198, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 221, 223, 225, 226, 231, 232, 233, 235, 239, 241, 257, 258, 259, and/or 261 of WO 2011/034631.

33. The method of claim 32, wherein at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: SEQ ID NOs 1-39, 176, 179, 180, 184, 188, 189, 190, 191, 193, 195, 198, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 221, 223, 225, 226, 231, 232, 233, 235, 239, 241, 257, 258, 259, and/or 261 of WO 2011/034631, *preferably* selected from the list consisting of: SEQ ID No NOs 33-39, 176, 179, 180, 184, 188, 189, 190, 191, 193, 195, 198, 199, 200, 201, 202, 203, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 221, 223, 225, 226, 231, 232, 233, 235, 239, 241, 257, 258, 259, and/or 261 of WO 2011/034631.

34. The method claim 14 or 31, wherein said DMR(s) comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and at least one of said DMR(s) is located in a region and/or gene disclosed in WO 2011/092592, including on selected from the list(s) consisting of: EP1, EP2, EP3, EP4, EP5, EP6, EP7, EP8, EP9, EP10, EP11 and EP12 [SEQ ID NOs 33-44] of WO 2011/092592.

35. The method claim 14 or 31, wherein said DMR(s) comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: AIRE, SIM2, ERG and VAPA-APCDDI, or is HLCS.

36. The method of any one of claims 14 or 31 to 35, wherein at least one of said DMR(s):

- is located on a human chromosome selected from the list consisting of: chromosome 21, chromosome 18, chromosome 13, X-chromosome and Y-chromosome, *preferably* chromosome 21 chromosome 18 or chromosome 13, *more preferably* chromosome 21; and/or
- comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and said DMR is located in a regions and/or gene selected from the list consisting of: maspin [preferably a portion of the maspin (aka "SERPINB5") gene that described in EP 1 751 307 as being differentially methylated between a foetus and its mother], CGI137, PDE9A, PPP1R2P2, Similarity to Fem1A (C.elegans), CGI009, CBR1, DSCAM, C21orf29 and CGI13.

37. The method of claim 14 to 31, wherein at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: RASSF1A, TBX3, ZFY, CDC42EP1, MGC15523, SOX14 and SPN.

38. The method of claim 32, wherein at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: SEQ ID NOs: 40-59 and 90-163 of WO 2011/034631.

39. The method of any one of claims 14, 31, 32, 37 or 38, wherein at least one of said DMR(s):

- is located on a human chromosome selected from the list consisting of: chromosome 1 to 12, chromosome 14 to 17, chromosome 19, chromosome 20 chromosome 22 and chromosome 23; and/or
- comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and said DMR is located in a regions and/or gene selected from the list consisting of: CD48, FAIM3, ARHGAP25, SELPLG, APC, CASP8, RARB, SCGB3A1, DAB2IP, PTPN6, THY1, TMEFF2 and PYCARD.

40. The method of any of claims 1 to 39, wherein a plurality of species of DNA are detected in said sample; *preferably wherein*, two species of DNA are detected in said sample.

41. The method of claim 40, wherein:

- in at least one detection step (b):
 - the presence of methylated DNA at a first DMR is used to indicate the presence of an amount of a first species of DNA in said sample and the absence of methylated DNA at said first DMR indicates the absence of said first species of DNA in said sample; *preferably wherein*, said first species of DNA originates from cells of a foetus and/or the placenta of a foetus, said sample is from a pregnant female and said first DMR is one set forth in any one of claims 33 to 36; and
 - the presence of methylated DNA at a second DMR is used to indicate the presence of an amount of a second species of DNA in said sample and the absence of methylated DNA at said second DMR indicates the absence of said second species of DNA in said sample; *preferably wherein*, said second species of DNA originates from cells of a foetus and/or the placenta of a foetus, said sample is from a pregnant female and said second DMR is one set forth any one of claims 37 to 39; and
- in at least one detection step (c):
 - a first amount of total DNA present in said sample is detected using a first region that is not differently methylated between said first species of DNA and the DNA not originating from cells of said type, the modification of which first other region by said reagent is insensitive to methylation of DNA, wherein said first other region is located between about 20bp and about 20kb upstream or downstream of said first DMR; and

- a second amount of total DNA present in said sample is detected using a second region that is not differently methylated between said second species of DNA and the DNA not originating from cells of said type, the modification of which second other region by said reagent is insensitive to methylation of DNA, wherein said second other region is located between about 20bp and about 20kb upstream or downstream of said second DMR.

42. The method of claim 41, wherein said detection in step (b) of said first and second DMRs and said detection in step (c) of said first and second other regions are made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously for such DMRs and other regions, and using: (x) a different detectable label(s) for each of said first and second DMRs; and (y) further different detectable label(s) for each of said first and second other regions.

43. The method of claim 42, wherein said detection in step (c) and said detection in step (b) are made by multiplex real-time quantitative probe-based PCR using at least one labelled probe specific for each of said DMRs and other regions.

44. The method of any one of claims 40 to 43, wherein said agent comprises at least one methylation sensitive restriction enzyme.

45. The method of any one of claims 41 to 44, wherein each of said detection steps comprises quantitative detection and said detected amount of each of said species of DNA is expressed as a relative concentration of said species of DNA to the respective amount total DNA detected in said sample from the respective other region.

46. The method of any one of claims 41 to 44, further comprising the steps:

- detecting an amount of total DNA in a standard sample of DNA of known amount using the same other regions as used in step (c); and
- comparing the signal detected from said standard sample of DNA to the respective signal detected in step (c) for each of the other regions.

47. The method of claim 46, wherein each of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as an absolute amount of each or said species of DNA in said sample.

48. The method of claim 45 or 47, further comprising the step:

- determining the relative amount, preferably a ratio, of: (x) said first species of DNA detected with the first DMR; and (y) said second species of DNA detected with the second DMR; and
- comparing said relative amount or ratio with a threshold and/or reference distribution of amount(s) or ratio(s), wherein: a relative amount or ratio that is higher or lower than said threshold and/or reference distribution of amount(s) or ratio(s) indicates the presence of an abnormality in said first and/or second species of DNA present in said sample; *preferably wherein*, said abnormality is a chromosomal abnormality; *more preferably wherein*, said chromosomal abnormality is associated with a foetal abnormality and/or congenital disorder; *yet more preferably wherein*, said chromosomal abnormality is selected from the group consisting of: a trisomy (such as trisomy 21, trisomy 18, or trisomy 13), a sex-

chromosome abnormality (such as Turners syndrome, Klinefelter syndrome, [Noonan syndrome,] Triple X syndrome, XXY syndrome, or Fragile X syndrome or XYY syndrome or XXYY syndrome), a chromosomal deletion (such as Prader-Willi syndrome, Cris-du-chat syndrome, Wolf-Hirschhorn syndrome, or 22q11 deletion syndrome, Duchene muscular dystrophy), Beckwith-Wiedemann syndrome, Canvan syndrome, and neurofibromatosis; *most preferable wherein*, said chromosomal abnormality is a trisomy, such as one selected from the list consisting of trisomy 21, trisomy 18, or trisomy 13.

49. **A method** for detecting a chromosomal aneuploidy in a foetus carried by a pregnant female, said method comprising the steps:

- (A) Determining, using a method as set forth in any one of the claims above, in a sample taken from said pregnant female the amount of a first species of DNA that originates from cells of a foetus and/or the placenta of a foetus, wherein said first species of DNA is located on a chromosome relevant to the chromosomal aneuploidy or within a section of a chromosome relevant to the chromosomal aneuploidy, and wherein said first species of DNA that originates from cells of a foetus and/or the placenta of a foetus is distinguished from its counterpart of maternal origin in the sample due to differential DNA methylation;
- (B) Determining, using a method as set forth in any one of the claims above, the amount of a second species of DNA that originates from cells of a foetus and/or the placenta of a foetus in said sample, wherein said second species of DNA is located on a reference chromosome, and wherein said second species of DNA that originates from cells of a foetus and/or the placenta of a foetus is distinguished from its counterpart of maternal origin in the sample due to differential DNA methylation;
- (C) determining the relative amount, preferable the ratio, of the amounts from (A) and (B); and
- (D) comparing said relative amount or ratio with a threshold and/or reference distribution of amount(s) or ratio(s), wherein: a relative amount or ratio that is higher or lower than said threshold and/or reference distribution of amount(s) or ratio(s) indicates the presence of the chromosomal aneuploidy in the foetus.

50. **A method** for detecting an increased risk of an individual suffering from or developing a medical condition; said method comprising the steps:

- (i) conducting the method of claim 24 or 26; and
 - (ii) comparing the amount of said species of DNA detected with a threshold amount and/or a reference distribution of amounts,
- wherein an increase in, or outlying of, the amount of said species of DNA indicates an increased risk of the individual suffering from or developing said medical condition

51. **A composition** comprising:

- one pair of PCR primers for amplifying said DMR as set forth in any of claims 1 to 50;
- one pair of PCR primers for amplifying said other region as set forth in any of claims 1 to 50;
- one labelled probe as set forth in claim 7; and
- one labelled probe as set forth in claim 8.

52. The composition of claim 51 further comprising:

- a further pair of PCR primers for amplifying a second DMR as set forth in any of claims 3 to 50; and a further labelled probe as set forth in claim 7 when back-referenced to claim 4; and/or
- a further pair of PCR primers for amplifying a second other region as set forth in any of claims 10 to 50; and a further labelled probe as set forth in claim 12.

53. **A kit** comprising:

- the primers and probes as set forth in claim 51 or 52; and
- *optionally*, further comprising: (i) a printed manual or computer readable memory comprising instructions to use said primers and probes to practice a method of any one of claims 1 to 50 and/or to produce or use the composition of claim 51 or 52; and/or (ii) one or more other item, component or reagent useful for the practice of a method of any one of claims 1 to 50 and/or the production or use of the composition of claim 51 or 52, including any such item, component or reagent disclosed herein, such as the reagent that differentially modifies methylated and non-methylated DNA as set forth in any one of claims 1 to 50.

54. **A computer program product** comprising: a computer readable medium encoded with a plurality of instructions for controlling a computing system to perform and/or manage an operation for determining: (x) an increased risk of an individual suffering from or developing a medical condition and/or (y) if a diagnosis for an anomaly in a species of DNA originating from cells of a given type may be performed, in each case from a sample from an individual comprising a species of DNA originating from cells of a given type in admixture with differently methylated DNA not originating from cells of said type, the DNA present in said sample being treated with a reagent that differentially modifies methylated and non-methylated DNA as set forth in any one of claims 1 to 50; said operation comprising the steps of:

- receiving: (i) one signal representing the quantitative detection of methylation at one or more DMRs as set forth in step (b) of any one of claims 1 to 50; and (ii) one signal representing the quantitative detection of total DNA using at least one other region as set forth in step (c) any of claims 1 to 50;
- determining a parameter from the signals (i) and (ii), wherein the parameter represents a quantitative amount of said species of DNA;
- comparing the parameter to with a threshold amount and/or reference distribution of amounts; and
- based on such comparison, determining a classification of whether, respectively, (x) an increased risk of an individual suffering from or developing a medical condition exists; and/or (y) a diagnosis for an anomaly in a species of DNA originating from cells of a given type may be performed.

55. The computer program product of claim 54, wherein said operation further comprises the steps:

- receiving a further signal representing the quantitative detection of total DNA in a standard sample of DNA as set forth in claim 25; and
- comparing said signal with the signal set forth in (ii) of claim 54, so as to determine said parameter that represents an absolute quantitative amount of said species of DNA.

56. The computer program product of claim 54 or 55, wherein said operation is for determining if a diagnosis for an anomaly in said species of DNA may be performed, and further comprises the step of determining from said parameter a number of random and/or enriched DNA molecules to be sequenced from, preferably from a separate aliquot of DNA of, said sample as part of said diagnosis.

57. The computer program product of claim 54 or 55, wherein said operation further comprises the steps:

- receiving: (i) one signal representing the quantitative detection of methylation at a second DMRs as set forth in step (b) of any one of claims 41 to 49; and (ii) one signal representing the quantitative detection of total DNA using a second other region as set forth in step (c) any of claims 41 to 49;

- determining a second parameter from the signals (i) and (ii), wherein the parameter represents a quantitative amount of said second species of DNA;
- determining the relative amount, preferable the ratio, of said parameter and said second parameter;
- comparing said relative amount or ratio with a threshold and/or reference distribution of amount(s) or ratio(s); and
- based on such comparison, determining a classification of whether an abnormality in said species of DNA or second species of DNA present in said sample; *preferably wherein*, said abnormality is a chromosomal abnormality; *more preferably wherein*, said chromosomal abnormality is associated with a foetal abnormality and/or congenital disorder; *yet more preferably wherein*, said chromosomal abnormality is selected from the group consisting of: a trisomy (such as trisomy 21, trisomy 18, or trisomy 13), a sex-chromosome abnormality (such as Turners syndrome, Klinefelter syndrome, [Noonan syndrome,] Triple X syndrome, XXY syndrome, or Fragile X syndrome or XYY syndrome or XXYY syndrome), a chromosomal deletion (such as Prader-Willi syndrome, Cris-du-chat syndrome, Wolf-Hirschhorn syndrome, or 22q11 deletion syndrome, Duchene muscular dystrophy), Beckwith-Wiedemann syndrome, Canvan syndrome, and neurofibromatosis; *most preferable wherein*, said chromosomal abnormality is a trisomy, such as one selected from the list consisting of trisomy 21, trisomy 18, or trisomy 13.

CLAIMS

1. **A method** for detecting in a sample from an individual an amount of a species of DNA originating from cells of a given type, which sample comprises said species of DNA in admixture with differently methylated DNA not originating from cells of said type; said method comprising the steps:
 - (a) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA;
 - (b) detecting in said sample the presence of methylation in said species of DNA at two or more differentially methylated regions (DMRs) that are differently methylated between said species of DNA and the DNA not originating from cells of said type, the modification of DNA of such DMRs by said reagent is sensitive to methylation of DNA, wherein the presence of methylated DNA at one or more of said DMRs indicates the presence of said amount of species of DNA in said sample and the absence of methylated DNA at said DMRs indicates the absence of said species of DNA in said sample; and
 - (c) detecting an amount of total DNA present in said sample using at least one other region that is not differently methylated between said species of DNA and the DNA not originating from cells of said type, the modification of which region(s) by said reagent is insensitive to methylation of DNA, wherein, said detection in step (b) and said detection in step (c) are made using the same aliquot of DNA of said sample, and in the same vessel, and effectively simultaneously for such DMRs and other region(s), and using: (x) the same detectable label(s) for each of said DMRs; and (y) a different detectable label(s) for said other region(s).
2. The method of claim 1, wherein said other region is located between about 20bp and about 20kb upstream or downstream of, and/or within the same gene as, at least one of said DMRs.
3. The method of claim 1 or 2, wherein said detection in step (c) comprises using at least two of said other regions; *preferably wherein*, the number of said other regions is the same as the number of DMRs used in step (b); *more preferably wherein*, one of said other regions is located between about 20bp and about 20kb upstream or downstream of a DMR used in step (b) and each other of the said other regions is located between about 20bp and about 20kb upstream or downstream of another of said DMRs.
4. The method of claim 3, wherein said detection in step (c) is made using: (x) the same detectable label(s) for each of said other regions or (y) a different detectable label(s) for each of said other regions.
5. The method of any one of claims 1 to 4, wherein said detection in step (c) and said detection in step (b) are made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously with each other, and by multiplex real-time quantitative probe-based PCR using at least one labelled probe specific for each of the said DMRs and other region(s).
6. The method any one of claims 1 to 5, wherein said species of DNA originates from cells of a foetus and/or the placenta of a foetus and said sample is from a pregnant female; *preferably wherein*, said species of DNA is circulating cell-free DNA and said sample is a blood fraction such as plasma or serum.

7. The method of any one of claims 1 to 5, wherein said species of DNA originates from a cell type associated with a medical condition; *preferably wherein*, said medical condition is one selected from the group consisting of: a cell proliferative disorder, an infection/infectious disease, a wasting disorder, a degenerative disorder, an (auto)immune disorder, kidney disease, liver disease, inflammatory disease acute toxicity, chronic toxicity, myocardial infarction, and a combination of any of the forgoing; *more preferably wherein*, said species of DNA is circulating cell-free DNA and said sample is a blood fraction such as plasma or serum.
8. The method of any one of claims 1 to 7, wherein said sample is a tissue sample or a sample of biological fluid; *preferably wherein*, said sample is a sample of biological fluid selected from the group consisting of: whole blood, a blood fraction, urine, saliva, sweat, ejaculate, tears, phlegm, vaginal secretion, vaginal wash and colonic wash; *more preferably wherein*, said sample is a plasma or serum sample.
9. The method of any one of claims 1 to 8, wherein said reagent that differentially modifies methylated and non-methylated DNA comprises an agent that selectively digests unmethylated over methylated DNA, *preferably wherein*, said agent comprises:
- at least one methylation sensitive enzyme;
 - at least one methylation sensitive restriction enzyme; and/or
 - an agent selected from the group consisting of: AatII, AclI, AclI, AfeI, AgeI, AgeI-HF, AscI, AsiSI, AvaI, BceAI, BmgBI, BsaAI, BsaHI, BsiEI, BsiWI, BsmBI, BspDI, BsrFI, BssHII, BstBI, BstUI, ClaI, EagI, FauI, FseI, FspI, HaeII, HgaI, HhaI, HinfI, HpaII, Hpy99I, HpyCH4IV, Kasi, MluI, NaeI, NarI, NgoMIV, NotI, NotI-HF, NruI, Nt.BsmAI, Nt.CviPII, PaeR7I, PluTI, PmlI, PvuI, PvuI-HF, RsrII, SacII, SalI, SalI-HF, SfoI, SgrAI, SmaI, SnaBI, TspMI and ZraI.
10. The method of any one of claims 1 to 9, wherein each of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as a relative concentration of said species of DNA to the total DNA in said sample.
11. The method of any one of claims 1 to 9, further comprising the steps:
- detecting an amount of total DNA in a standard sample of DNA of known amount using the same other regions(s) as used in step (c); and
 - comparing the signal detected from said standard sample of DNA to the signal detected in step (c).
12. The method of claim 11, wherein each of said detection steps comprises quantitative detection and said detected amount of said species of DNA is expressed as an absolute amount of said species of DNA in said sample.
13. The method of claim 6, wherein said DMR(s) is/are hypermethylated in foetal DNA and hypo methylated in maternal DNA.
14. The method of claim 6 or 13, wherein at least one of said DMR(s):
- is located on a human chromosome selected from the list consisting of: chromosome 21, chromosome 18, chromosome 13, X-chromosome and Y-chromosome, *preferably* chromosome 21, chromosome 18, chromosome 13, *most preferably* chromosome 21; and/or

- comprises at least one, preferably at least two, methylation site(s) specific for said reagent, and said DMR is located in a regions and/or gene selected from the list consisting of: maspin [preferably a portion of the maspin (aka "SERPINB5") gene that described in EP 1 751 307 as being differentially methylated between a foetus and its mother], CGI137, PDE9A, PPP1R2P2, Similarity to Fem1A (C.elegans), CGI009, CBR1, DSCAM, C21orf29 and CGI13.

15. The method of claim 6 or 13, wherein at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: RASSF1A, TBX3, ZFY, CDC42EP1, MGC15523, SOX14 and SPN.

16. The method of any of claims 1 to 15, wherein a plurality of species of DNA are detected in said sample; *preferably wherein*, two species of DNA are detected in said sample.

17. **A method** for detecting a chromosomal aneuploidy in a foetus carried by a pregnant female, said method comprising the steps:

- Determining, using a method as set forth in any one of the preceding claims, in a sample taken from said pregnant female the amount of a first species of DNA that originates from cells of a foetus and/or the placenta of a foetus, wherein said first species of DNA is located on a chromosome relevant to the chromosomal aneuploidy or within a section of a chromosome relevant to the chromosomal aneuploidy, and wherein said first species of DNA that originates from cells of a foetus and/or the placenta of a foetus is distinguished from its counterpart of maternal origin in the sample due to differential DNA methylation;
- Determining, using a method as set forth in any one of the preceding claims, the amount of a second species of DNA that originates from cells of a foetus and/or the placenta of a foetus in said sample, wherein said second species of DNA is located on a reference chromosome, and wherein said second species of DNA that originates from cells of a foetus and/or the placenta of a foetus is distinguished from its counterpart of maternal origin in the sample due to differential DNA methylation;
- determining the relative amount, preferable the ratio, of the amounts from (A) and (B); and
- comparing said relative amount or ratio with a threshold and/or reference distribution of amount(s) or ratio(s), wherein: a relative amount or ratio that is higher or lower than said threshold and/or reference distribution of amount(s) or ratio(s) indicates the presence of the chromosomal aneuploidy in the foetus.

18. **A method** for detecting an increased risk of an individual suffering from or developing a medical condition; said method comprising the steps:

- conducting the method of claim 10 or 12; and
- comparing the amount of said species of DNA detected with a threshold amount and/or a reference distribution of amounts,

wherein an increase in, or outlying of, the amount of said species of DNA indicates an increased risk of the individual suffering from or developing said medical condition.

19. **A composition** comprising:

- two pairs of PCR primers, each pair for amplifying one of said two of more DMRs as set forth in any of claim 1 to 18;
- one pair of PCR primers for amplifying said other region as set forth in any of claims 1 to 18;
- two labelled probes each of which specific for one of said DMRs; and

- one labelled probe specific for one of said other regions(s).

20. **A kit** comprising:

- the primers and probes: as set forth in claim 19; and
- *optionally*, further comprising: (i) a printed manual or computer readable memory comprising instructions to use said primers and probes to practice a method of any one of claims 1 to 18 and/or to produce or use the composition of claim 19; and/or (ii) one or more other claim, component or reagent useful for the practice of a method of any one of claims 1 to 18 and/or the production or use of the composition of claim 19, including any such claim, component or reagent disclosed herein, such as the reagent that differently modifies methylated and non-methylated DNA as set forth in any one of claims 1 to 18.

21. **A computer program product** comprising a computer readable medium encoded with a plurality of instructions for controlling a computing system to perform and/or manage an operation for determining: (x) an increased risk of an individual suffering from or developing a medical condition and/or (y) if a diagnosis for an anomaly in a species of DNA originating from cells of a given type may be performed, in each case from a sample from an individual comprising a species of DNA originating from cells of a given type in admixture with differently methylated DNA not originating from cells of said type, the DNA in present in said sample being treated with a reagent that differentially modifies methylated and non-methylated DNA as set forth in any one of claims 1 to 18; said operation comprising the steps of:

- receiving: (i) one signal representing the essentially simultaneous quantitative detection of methylation at two or more DMRs as set forth in step (b) of any one of claims 1 to 18; and (ii) one signal representing the essentially simultaneous quantitative detection of total DNA using at least one other region as set forth in step (c) any of claims 1 to 18;
- determining a parameter from the signals (i) and (ii), wherein the parameter represents a quantitative amount of said species of DNA;
- comparing the parameter to with a threshold amount and/or reference distribution of amounts; and
- based on such comparison, determining a classification of whether, respectively, (x) an increased risk of an individual suffering from or developing a medical condition exists; and/or (y) a diagnosis for an anomaly in a species of DNA originating from cells of a given type may be performed.

22. **A method** for detecting in a sample from an individual an amount of a species of DNA originating from cells of a given type, which sample comprises said species of DNA in admixture with differentially methylated DNA not originating from cells of said type; said method comprising the steps:

- (a) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA; and
- (b) detecting in said sample the presence of methylation in said species of DNA at two or more DMRs that are differently methylated between said species of DNA and the DNA not originating from cells of said type the modification of DNA of such DMRs by said reagent is sensitive to methylation of DNA, wherein the presence of methylated DNA at one or more of said DMRs indicates the presence of said amount of species of DNA in said sample and the absence of methylated DNA at said DMRs indicates the absence of said species of DNA in said sample,

wherein, said detection in step (b) is made using the same aliquot of DNA of said sample, and in the same reaction/detection vessel, and effectively simultaneously for such DMRs, and using (x) multiplex real-time

quantitative PCR; and (y) at least two labelled probes each of which specific for one of said DMRs and that are labelled with the same detectable label(s) for each of said DMRs; *preferably wherein*, said reagent comprises agent as set forth in claim 9.