

## CLAIMS

1. **A method** for quantitatively 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, 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;  
said method comprising the steps:
  - (a) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA, wherein said reagent comprises at least one methylation sensitive restriction enzyme;
  - (b) quantitatively 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 wherein said DMRs are hyper-methylated in foetal DNA and hypo-methylated in maternal DNA; and
  - (c) quantitatively detecting an amount of total DNA present in said sample using at least one other region in the human genome that does not comprise a site of methylation recognised by said methylation sensitive restriction enzyme,

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 labels(s) for each of said DMRs; and (y) a different detectable label(s) for said other region(s); and

wherein:

  - (A) as part of said detection steps (b) and (c) each DNA region comprising said DMRs and other regions(s) are amplified; and
  - (B) each detectable label used in step (b) and (c) is a fluorescent label; and
  - (C) said detection in step (b) comprises multiplex real-time quantitative probe-based PCR using at least two labelled probes each of which specific for one of said DMRs; and
  - (D) 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).
2. The method of claim 1, wherein two of said DMRs are located on different chromosomes.
3. The method of claim 1 or 2, 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.
4. The method of any one of claims 1 to 3, wherein said detection in step (c) comprises using at least two of said other regions; *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.

5. The method of claim 4, wherein said detection in step (c) is made using the same detectable label(s) for each of said other regions.
6. The method of claim 4 or 5, wherein said detection in step (c) comprises multiplex real-time quantitative probe-based PCR using at least two labelled probes each of which is specific for one of said other regions.
7. The method of any one of claims 1 to 6, 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).
8. The method any one of claims 1 to 7, wherein said species of DNA is circulating cell-free DNA and said sample is a plasma or serum sample.
9. The method of any one of claims 1 to 8, wherein said DMRs comprises at least one, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, and at least one 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.
10. The method of any one of claims 1 to 9, wherein said other region is located in a portion of the genome and/or gene selected from the group consisting of: GAPDH, beta-actin, ALB, APOE, RNASEP, RASSF1A, TBX3, HLCS, ZFY, CDC42EP1, MGC15523, SOX14 and SPN.
11. The method of any one of claims 1 to 10, wherein:  
(A) at least one of said DMRs is located between positions 1,660bp and 2,400bp of TBX3; and/or  
(B) said other region is located between positions 12,400bp and 13,000bp of TBX3.
12. The method of any one of claims 1 to 11, 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.
13. The method of claim 12, wherein said pregnancy-associated medical condition is preeclampsia.
14. The method of any one of claims 1 to 13, wherein said sample is a tissue sample or a sample of biological fluid from the group consisting of: whole blood, a blood fraction, urine, saliva, sweat, tears, phlegm, vaginal secretion, vaginal wash and colonic wash.
15. The method of claim 14, wherein the sample is a plasma or serum sample.
16. The method of any one of claims 1 to 15, wherein said reagent comprises a methylation sensitive enzyme
  - selected from the group consisting of: AatII, AciI, 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, HinP1I, 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.

- 5 17. The method of claim 16, wherein said reagent comprises two or three of the methylation sensitive restriction enzymes selected from the group consisting of BstUI, HhaI and HpaII.
18. The method of any one of claims 1 to 17, 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.
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19. The method of any one of claims 1 to 17, 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
  - 15 • comparing the signal detected from said standard sample of DNA to the signal detected in step (c).
20. The method of claim 19, 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.
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21. The method of claim 18 or 20, 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 said sample.
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22. The method of any one of claims 18 to 21, further comprising the step:
- 30 • performing on said sample, a diagnosis for an abnormality in said species of DNA present in said sample; *wherein*, said diagnosis is a prenatal diagnosis.
23. The method of claim 22, 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.
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24. The method of claim 22 or 23, wherein:
- 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
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- 5       •     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, Fragile X syndrome, XYY syndrome, or XYYY 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.
- 10     25.   The method of claim 24, wherein said chromosomal abnormality is a chromosomal trisomy that is trisomy 21, trisomy 18, or trisomy 13.
- 15     26.   The method of any one of claims 1 to 8, wherein said DMR(s) comprises at least one, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, and at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of SEQ ID NOs 15 to 187.
- 20     27.   The method of any one of claims 1 to 26, wherein at least one of said DMRs is located on human chromosome 12.
- 25     28.   The method of claim 25, 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 15 to 53, 148 to 187.
- 30     29.   The method of any one of claims 1 to 8, wherein said DMR(s) comprises at least one, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, and at least one of said DMR(s) is located in a region and/or gene selected from the list consisting of: SEQ ID Nos 188 to 199.
- 35     30.   The method of any one of claims 1 to 8, wherein said DMR(s) comprises at least one, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, 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.
- 40     31.   The method of any one of claims 1 to 8, or 28 to 30, wherein at least one of said DMR(s):
 
  - is located on a human chromosome selected from the list consisting of: chromosome 21, chromosome 18, and chromosome 13; and/or
  - comprises at least one, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, and said DMR is located in a region and/or gene selected from the list consisting of: maspin, CGI137, PDE9A, PPP1R2P2, CGI009, CBR1, DSCAM, C21orf29 and CGI13.
- 45     32.   The method of any one of claims 1 to 8, 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.
- 50     33.   The method of any one of claims 1 to 8, 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: 54 to 147.

34. The method of any one of claims 1 to 8, 26, 27, 32 or 33, 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, or at least two, site(s) of methylation recognised by said methylation sensitive restriction enzyme, and said DMR is located in a region and/or gene selected from the list consisting of: CD48, FAIM3, ARHGAP25, SELPLG, APC, CASP8, RARB, SCGB3A1, DAB2IP, PTPN6, THY1, TMEFF2 and PYCARD.

35. The method of any of claims 1 to 34, wherein two species of DNA are detected in said sample.

36. **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.

37. **A method** for detecting an increased risk of an individual suffering from or developing a medical condition, wherein the individual is a pregnant female and said medical condition is preeclampsia; said method comprising the steps:

- (i) conducting the method of claim 18 or 20 to quantitatively detecting in a sample from said pregnant female an amount of a species of DNA originating from cells of a foetus and/or the placenta of a foetus; 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 pregnant female suffering from or developing preeclampsia.

38. **A composition** comprising:

- (A) two pairs of PCR primers, each pair designed to amplify one of two first regions comprising at least one, or at least two, site(s) of methylation recognised by a methylation sensitive restriction enzyme, each

first region located in a region and/or gene independently selected from the list consisting of: SEQ ID Nos 15 to 187, or located in a region and/or gene independently selected from the list consisting of: SEQ ID Nos 188 to 199;

- (B) two fluorescently labelled real-time quantitative PCR probes each of which specific for one of said first regions of (A) and that have the same fluorescent label(s);
- (C) one pair of PCR primers designed to amplify an other region in the human genome, wherein said other region does not comprise a recognition sequence for the methylation sensitive restriction enzyme; *preferably wherein* said other region is located between 20bp and 20kb upstream or downstream of at least one of said first regions of (A); and
- (D) one fluorescently labelled real-time quantitative PCR probe which is specific for said other region and that has a different fluorescent label(s) than that of the fluorescently labelled probes which are specific for said first regions of (A).

39. The composition of claim 38, further comprising:

- (E) a further pair of PCR primers designed to amplify a second other region in the human genome, wherein said second other region does not comprise a recognition sequence for the methylation sensitive restriction enzyme; *preferably wherein* said second other region is located between 20bp and 20kb upstream or downstream of another of said first regions of (A); and
- (F) a further fluorescently labelled real-time quantitative PCR probe which is specific for said second other region and that has a different fluorescent label(s) than that of the fluorescently labelled probes which are specific for said first regions of (A).

40. The composition of claim 38 or 39, wherein one other region is located between positions 12,400bp and 13,000bp of TBX3.

41. The composition of any one of claims 38 to 40, wherein said two first regions of (A) are located on different chromosomes.

42. The composition of any one of claims 38 to 41, wherein at least one of said first regions of (A) is located on human chromosome 12.

43. **A kit** comprising:

- the primers and probes as set forth in any one of claims 38 to 42.

44. The kit of claim 43 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 27, 29 or 37 and/or to produce or use the composition of any one of claims 38 to 42; 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 27, 29 or 37 and/or the production or use of the composition of any one of claims 38 to 42.

45. **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 and 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, the DNA in present in said sample being treated with a reagent that differentially modifies methylated and non-methylated DNA, wherein said reagent comprises at least one methylation sensitive restriction enzyme;;  
said operation comprising the steps of:

- receiving: (i) one signal from real-time quantitative probe-based PCR 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 27, 29 or 37; and (ii) one signal from real-time quantitative probe-based PCR representing the essentially simultaneous quantitative detection of total DNA using at least one other region as set forth in step (c) of any of claims 1 to 27, 29 or 37;
- determining a parameter from the signals (i) and (ii), wherein the parameter represents a quantitative amount of said species of DNA;
- comparing the parameter 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 the species of DNA originating from the cells of a foetus and/or the placenta of the foetus may be performed.

46. The computer program product of claim 45, 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 18; and
- comparing said signal with the signal set forth in (ii) of claim 45, so as to determine said parameter that represents an absolute quantitative amount of said species of DNA.

47. The computer program product of claim 45 or 46, wherein said operation is for determining if a diagnosis for an anomaly in said species of DNA may be performed.

48. The computer program product of any one of claims 45 to 47, 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.

49. The computer program product of any one of claims 45 to 48, wherein said species of DNA is circulating cell-free DNA and said sample is a plasma or serum sample.

50. The computer program product of any one of claims 45 to 49, wherein a classification of whether a prenatal diagnosis may be performed for an anomaly in the DNA originating from cells of a foetus and/or the placenta of a foetus is determined.

51. The computer program product of claim 50, wherein said anomaly in the DNA originating from cells of a foetus and/or the placenta of a foetus is a chromosomal trisomy.

52. The computer program product of any one of claims 45 to 49, wherein a classification of whether there is an increased risk of said pregnant female suffering from 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 is determined.

53. The computer program product of claim 52, wherein said pregnancy-associated medical condition is preeclampsia.

54. **A method** for quantitatively 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, 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; said method comprising the steps:

(a) treating the DNA present in said sample with a reagent that differentially modifies methylated and non-methylated DNA, wherein said reagent comprises at least one methylation sensitive restriction enzyme; and

(b) quantitatively 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, and wherein said DMRs are hyper-methylated in foetal DNA and hypo methylated in maternal DNA,

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; and

wherein:

(A) as part of said detection step (b) each DNA region comprising said DMRs are amplified; and

(B) each detectable label used in step (b) is a fluorescent label; and

(C) said detection in step (b) comprises multiplex real-time quantitative probe-based PCR using at least two labelled probes each of which specific for one of said DMRs.