

WHAT IS CLAIMED IS:

1. A method of diagnosing a pregnancy-associated disorder using a sample from a female subject pregnant with a fetus, the method comprising:

receiving a plurality of reads, wherein the reads are obtained from an analysis of RNA molecules obtained from the sample, the sample containing a mixture of maternal- and fetal-derived RNA molecules;

identifying, by a computer system, locations of the reads in a reference sequence;

identifying one or more informative loci, each of which is homozygous in a first entity for a corresponding first allele and which is heterozygous in a second entity for the corresponding first allele and a corresponding second allele, wherein the first entity is the pregnant female subject or the fetus, and the second entity is the other one of the pregnant female subject and the fetus;

filtering the one or more informative loci to identify one or more filtered informative loci:

that are located within an expressed region of the reference sequence, at which at least a first predetermined number of reads in the plurality of reads containing the corresponding first allele are located, and at which at least a second predetermined number of reads in the plurality of reads containing the corresponding second allele are located,

for each of the filtered informative loci:

determining a first number of reads located at the filtered informative locus and containing the corresponding first allele,

determining a second number of reads located at the filtered informative locus and containing the corresponding second allele,

calculating a first sum of the first numbers and the second sum of the second numbers;

calculating a ratio of the first sum and the second sum, and

comparing the ratio to a cutoff value to determine whether the fetus, mother, or pregnancy has a pregnancy-associated disorder, the cutoff value being determined from one or more samples obtained from pregnant female subjects without the pregnancy-associated disorder.

- 1 2. The method of claim 1, wherein the sample of the pregnant woman is a
2 sample of blood plasma.
- 1 3. The method of claim 1, wherein the second entity is the pregnant
2 female subject.
- 1 4. The method of claim 1, wherein the second entity is the fetus.
- 1 5. The method of claim 1, wherein calculating a ratio of the first sum and
2 the second sum comprises dividing the second sum by a sum of the first sum and the second
3 sum.
- 1 6. The method of claim 1, wherein the analysis of RNA molecules
2 obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.
- 1 7. The method of claim 1, wherein the analysis of RNA molecules
2 obtained from the sample comprises performing digital PCR.
- 1 8. The method of claim 1, wherein identifying locations of the reads in a
2 reference sequence comprises aligning the reads to the reference sequence.
- 1 9. The method of claim 1, wherein the first predetermined number of
2 reads is 1, and wherein the second predetermined number of reads is 1.
- 1 10. The method of claim 1, further comprising sequencing genomic DNA
2 obtained from maternal tissue to determine a genotype of the pregnant female subject at each
3 informative locus.
- 1 11. The method of claim 1, further comprising sequencing genomic DNA
2 obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a
3 genotype of the fetus at each informative locus.
- 1 12. The method of claim 1, further comprising estimating a portion of
2 RNA in the sample that is of maternal or fetal origin, wherein the portion is the ratio
3 multiplied by a scalar.
- 1 13. A method of determining, in a sample from a female subject pregnant
2 with a fetus, a portion of RNA that is of fetal origin, the method comprising:

3 receiving a plurality of reads, wherein the reads are obtained from an analysis
4 of RNA molecules obtained from the sample, the sample containing a mixture of maternal-
5 and fetal-derived RNA molecules;

6 identifying, by a computer system, locations of the reads in a reference
7 sequence;

8 identifying one or more informative maternal loci, each of which is
9 homozygous in the fetus for a corresponding first allele and which is heterozygous in the
10 pregnant female subject for the corresponding first allele and a corresponding second allele;

11 filtering the one or more informative maternal loci to identify one or more
12 filtered informative maternal loci:

13 that are located within an expressed region of the reference sequence,
14 at which at least a first predetermined number of reads in the plurality of
15 reads containing the corresponding first allele are located, and

16 at which at least a second predetermined number of reads in the plurality
17 of reads containing the corresponding second allele are located,

18 for each of the filtered informative maternal loci:

19 determining a first number of reads located at the filtered informative
20 maternal locus and containing the corresponding first allele,

21 determining a second number of reads located at the filtered informative
22 maternal locus and containing the corresponding second allele,

23 calculating a sum of the first number and the second number,

24 calculating a maternal ratio of the second number divided by the sum,

25 determining a scalar representing a total expression at the filtered
26 informative maternal locus relative to expression of the corresponding second allele in the
27 pregnant female subject,

28 multiplying the maternal ratio by the scalar to obtain a maternal
29 contribution,

30 calculating a fetal contribution as one minus the maternal contribution, and

31 determining a portion of RNA in the sample that is of fetal origin, wherein the
32 portion is an average of the fetal contributions for the filtered informative maternal loci.

1 14. The method of claim 13, wherein the average is weighted by the sums
2 for the filtered informative maternal loci.

1 15. The method of claim 13, wherein the scalar is assumed to be about 2
2 for a filtered informative maternal locus.

1 16. The method of claim 13, wherein the sample of the pregnant woman is
2 a sample of blood plasma.

1 17. The method of claim 13, wherein the analysis of RNA molecules
2 obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.

1 18. The method of claim 13, wherein the analysis of RNA molecules
2 obtained from the sample comprises performing digital PCR.

1 19. The method of claim 13, wherein identifying locations of the reads in a
2 reference sequence comprises aligning the reads to the reference sequence.

1 20. The method of claim 13, wherein the first predetermined number of
2 reads for the one or more filtered informative maternal loci is 1, and wherein the second
3 predetermined number of reads for the one or more filtered informative maternal loci is 1.

1 21. The method of claim 13, further comprising sequencing genomic DNA
2 obtained from maternal tissue to determine a genotype of the pregnant female subject at each
3 informative maternal locus.

1 22. The method of claim 13, further comprising sequencing genomic DNA
2 obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a
3 genotype of the fetus at each informative maternal locus.

1 23. The method of claim 13, further comprising:
2 identifying one or more informative fetal loci, each of which is homozygous in
3 the pregnant female subject for a corresponding first allele and which is heterozygous in the
4 fetus for the corresponding first allele and a corresponding second allele;

5 filtering the one or more informative fetal loci to identify one or more filtered
6 informative fetal loci:

7 that are located within an expressed region of the reference sequence,
8 at which at least a first predetermined number of reads in the plurality of
9 reads containing the corresponding first allele are located, and

10 at which at least a second predetermined number of reads in the plurality
11 of reads containing the corresponding second allele are located,
12 for each of the filtered informative fetal loci:
13 determining a first number of reads located at the filtered informative fetal
14 locus and containing the corresponding first allele,
15 determining a second number of reads located at the filtered informative
16 fetal locus and containing the corresponding second allele,
17 calculating a sum of the first number and the second number,
18 calculating a fetal ratio of the second number divided by the sum,
19 determining a scalar representing a total expression at the filtered
20 informative fetal locus relative to expression of the corresponding second allele in the
21 fetus;
22 multiplying the fetal ratio by the scalar to obtain a fetal contribution, and
23 determining a portion of RNA in the sample that is of fetal origin, wherein the
24 portion is the average of the fetal contributions for the filtered informative maternal loci and
25 the filtered informative fetal loci.

1 24. The method of claim 23, wherein the average is weighted by the sums
2 for the filtered informative maternal loci and the filtered informative fetal loci.

1 25. The method of claim 23, wherein the scalar is assumed to be about 2
2 for a filtered informative fetal locus.

1 26. The method of claim 23, wherein the first predetermined number of
2 reads for the one or more filtered informative fetal loci is 1 and the second predetermined
3 number of reads for the one or more filtered informative fetal loci is 1.

1 27. The method of claim 23, further comprising sequencing genomic DNA
2 obtained from maternal tissue to determine a genotype of the pregnant female subject at each
3 informative fetal locus.

1 28. The method of claim 23, further comprising sequencing genomic DNA
2 obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a
3 genotype of the fetus at each informative fetal locus.

1 29. The method of claim 13 or 23, further comprising diagnosing a
2 pregnancy-associated disorder by comparing the portion of RNA in the sample that is of fetal
3 origin to a cutoff value.

1 30. A method of designating a genomic locus as a maternal or fetal marker
2 by analyzing a sample from a female subject pregnant with a fetus, the method comprising:
3 receiving a plurality of reads, wherein the reads are obtained from an analysis
4 of RNA molecules obtained from the sample, the sample containing a mixture of maternal-
5 and fetal-derived RNA molecules;

6 identifying, by a computer system, locations of the reads in a reference
7 sequence;

8 identifying one or more informative loci, each of which is homozygous in a
9 first entity for a corresponding first allele and which is heterozygous in a second entity for the
10 corresponding first allele and a corresponding second allele, wherein the first entity is the
11 pregnant female subject or the fetus, and the second entity is the other one of the pregnant
12 female subject and the fetus;

13 filtering the one or more informative loci to identify one or more filtered
14 informative loci:

15 that are located within an expressed region of the reference sequence,
16 at which at least a first predetermined number of reads in the plurality of
17 reads containing the corresponding first allele are located, and

18 at which at least a second predetermined number of reads in the plurality
19 of reads containing the corresponding second allele are located,

20 for each of the filtered informative loci:

21 determining a first number of reads located at the filtered informative
22 locus and containing the corresponding first allele,

23 determining a second number of reads located at the filtered informative
24 locus and containing the corresponding second allele,

25 calculating a ratio of the first number and the second number,

26 designating the filtered informative locus as a marker for the second entity
27 when the ratio exceeds a cutoff.

1 31. The method of claim 30, wherein the sample of the pregnant woman is
2 a sample of blood plasma.

- 1 32. The method of claim 30, wherein the second entity is the pregnant
2 female subject.
- 1 33. The method of claim 30, wherein the second entity is the fetus.
- 1 34. The method of claim 30, wherein calculating a ratio of the first number
2 and the second number comprises dividing the second number the sum of the first number
3 and the second number.
- 1 35. The method of claim 30, wherein the cutoff is from about 0.2 to about
2 0.5.
- 1 36. The method of claim 35, wherein the cutoff is 0.4.
- 1 37. The method of claim 30, wherein the analysis of RNA molecules
2 obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.
- 1 38. The method of claim 30, wherein the analysis of RNA molecules
2 obtained from the sample comprises performing digital PCR.
- 1 39. The method of claim 30, wherein identifying locations of the reads in a
2 reference sequence comprises aligning the reads to the reference sequence.
- 1 40. The method of claim 30, wherein the first predetermined number of
2 reads is 1 and the second predetermined number of reads is 1.
- 1 41. The method of claim 30, further comprising sequencing genomic DNA
2 obtained from maternal tissue to determine a genotype of the pregnant female subject at each
3 informative locus.
- 1 42. The method of claim 30, further comprising sequencing genomic DNA
2 obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a
3 genotype of the fetus at each informative locus.
- 1 43. The method of claim 30, further comprising diagnosing a pregnancy-
2 associated disorder based upon whether a filtered informative locus is designated as a marker
3 for the second entity.

1 44. The method of claim 30, further comprising determining, for RNA in
2 the sample that contains a filtered informative locus, a portion of the RNA that originates
3 from the second entity, wherein the portion is determined by multiplying the ratio by a scalar.

1 45. The method of claim 44, wherein the scalar represents a total
2 expression at the filtered informative maternal locus relative to expression of the
3 corresponding second allele in the second entity.

1 46. The method of claim 44, wherein the scalar is assumed to be about 2.

1 47. The method of claim 44, further comprising determining a portion of
2 the RNA that originates from the first entity by subtracting the portion of RNA that originates
3 from the second entity from 1.

1 48. Use of a gene listed in FIG. 21 or FIG. 26 for diagnosing a pregnancy-
2 associated disorder in a female subject pregnant with a fetus by comparing an expression
3 level of the gene with a control value determined from one or more other female subjects,
4 each pregnant with a healthy fetus.

1 49. The use of claim 48, wherein the medical disorder is associated with
2 the female subject.

1 50. The use of claim 48, wherein the medical disorder is associated with
2 the fetus.

1 51. The use of claim 50, wherein the medical disorder is pre-eclampsia or
2 pre-term birth.

1 52. The use of claim 48, wherein the medical disorder is diagnosed if the
2 expression level exceeds the control value.

1 53. The use of claim 48, wherein the medical disorder is diagnosed if the
2 expression level is below the control value.

1 54. The use of claim 48, wherein the female subject exhibits an elevated
2 expression level of the gene while pregnant as compared with after delivery.

1 55. The use of claim 48, wherein the expression level of the gene in a
2 female subject is measured by
3 obtaining a blood plasma sample from the female subject,
4 receiving a plurality of reads, wherein the reads are obtained from an analysis
5 of RNA molecules obtained from the sample;
6 identifying, by a computer system, locations of the reads in a reference
7 sequence; and
8 counting the reads located at the gene.

1 56. A system comprising one or more processors configured to perform
2 any of the above methods.

1 57. A computer product comprising a computer readable medium storing a
2 plurality of instructions for controlling a processor to perform an operation of any of the
3 methods above.

WHAT IS CLAIMED IS:

1. An ex vivo method of diagnosing a pregnancy-associated disorder using a sample from a female subject pregnant with a fetus, the method comprising:
 - receiving a plurality of reads, wherein the reads are obtained from an analysis of RNA molecules obtained from the sample, the sample containing a mixture of maternal- and fetal-derived RNA molecules;
 - identifying, by a computer system, locations of the reads in a reference sequence;
 - identifying one or more informative loci, each of which is homozygous in a first entity for a corresponding first allele and which is heterozygous in a second entity for the corresponding first allele and a corresponding second allele, wherein the first entity is the pregnant female subject or the fetus, and the second entity is the other one of the pregnant female subject and the fetus, wherein the one or more informative loci are identified using a sample from the female subject;
 - filtering the one or more informative loci to identify one or more filtered informative loci:
 - that are located within an expressed region of the reference sequence,
 - at which at least a first predetermined number of reads in the plurality of reads containing the corresponding first allele are located, and
 - at which at least a second predetermined number of reads in the plurality of reads containing the corresponding second allele are located,
 - for each of the filtered informative loci:
 - determining a first number of reads located at the filtered informative locus and containing the corresponding first allele,
 - determining a second number of reads located at the filtered informative locus and containing the corresponding second allele,
 - calculating a first sum of the first numbers and the second sum of the second numbers;
 - calculating a ratio of the first sum and the second sum, and
 - comparing the ratio to a cutoff value to determine whether the fetus, mother, or pregnancy has a pregnancy-associated disorder, the cutoff value being determined from one or more samples obtained from pregnant female subjects without the pregnancy-associated disorder.

2. The method of claim 1, wherein the sample of the pregnant woman is a sample of blood plasma.
3. The method of claim 1, wherein the second entity is the pregnant female subject.
4. The method of claim 1, wherein the second entity is the fetus.
5. The method of claim 1, wherein calculating a ratio of the first sum and the second sum comprises dividing the second sum by a sum of the first sum and the second sum.
6. The method of claim 1, wherein the analysis of RNA molecules obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.
7. The method of claim 1, wherein the analysis of RNA molecules obtained from the sample comprises performing digital PCR.
8. The method of claim 1, wherein identifying locations of the reads in a reference sequence comprises aligning the reads to the reference sequence.
9. The method of claim 1, wherein the first predetermined number of reads is 1, and wherein the second predetermined number of reads is 1.
10. The method of claim 1, further comprising sequencing genomic DNA obtained from maternal tissue to determine a genotype of the pregnant female subject at each informative locus.
11. The method of claim 1, further comprising sequencing genomic DNA obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a genotype of the fetus at each informative locus.
12. The method of claim 1, further comprising estimating a portion of RNA in the sample that is of maternal or fetal origin, wherein the portion is the ratio multiplied by a scalar.
13. A method of determining, in a sample from a female subject pregnant with a fetus, a portion of RNA that is of fetal origin, the method comprising:

receiving a plurality of reads, wherein the reads are obtained from an analysis of RNA molecules obtained from the sample, the sample containing a mixture of maternal- and fetal-derived RNA molecules;

identifying, by a computer system, locations of the reads in a reference sequence;

identifying one or more informative maternal loci, each of which is homozygous in the fetus for a corresponding first allele and which is heterozygous in the pregnant female subject for the corresponding first allele and a corresponding second allele;

filtering the one or more informative maternal loci to identify one or more filtered informative maternal loci:

that are located within an expressed region of the reference sequence,

at which at least a first predetermined number of reads in the plurality of reads containing the corresponding first allele are located, and

at which at least a second predetermined number of reads in the plurality of reads containing the corresponding second allele are located,

for each of the filtered informative maternal loci:

determining a first number of reads located at the filtered informative maternal locus and containing the corresponding first allele,

determining a second number of reads located at the filtered informative maternal locus and containing the corresponding second allele,

calculating a sum of the first number and the second number,

calculating a maternal ratio of the second number divided by the sum,

determining a scalar representing a total expression at the filtered informative maternal locus relative to expression of the corresponding second allele in the pregnant female subject,

multiplying the maternal ratio by the scalar to obtain a maternal contribution,

calculating a fetal contribution as one minus the maternal contribution, and

determining a portion of RNA in the sample that is of fetal origin, wherein the portion is an average of the fetal contributions for the filtered informative maternal loci.

14. The method of claim 13, wherein the average is weighted by the sums for the filtered informative maternal loci.

15. The method of claim 13, wherein the scalar is assumed to be about 2 for a filtered informative maternal locus.

16. The method of claim 13, wherein the sample of the pregnant woman is a sample of blood plasma.

17. The method of claim 13, wherein the analysis of RNA molecules obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.

18. The method of claim 13, wherein the analysis of RNA molecules obtained from the sample comprises performing digital PCR.

19. The method of claim 13, wherein identifying locations of the reads in a reference sequence comprises aligning the reads to the reference sequence.

20. The method of claim 13, wherein the first predetermined number of reads for the one or more filtered informative maternal loci is 1, and wherein the second predetermined number of reads for the one or more filtered informative maternal loci is 1.

21. The method of claim 13, further comprising sequencing genomic DNA obtained from maternal tissue to determine a genotype of the pregnant female subject at each informative maternal locus.

22. The method of claim 13, further comprising sequencing genomic DNA obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a genotype of the fetus at each informative maternal locus.

23. The method of claim 13, further comprising:
identifying one or more informative fetal loci, each of which is homozygous in the pregnant female subject for a corresponding first allele and which is heterozygous in the fetus for the corresponding first allele and a corresponding second allele;
filtering the one or more informative fetal loci to identify one or more filtered informative fetal loci:
that are located within an expressed region of the reference sequence,
at which at least a first predetermined number of reads in the plurality of reads containing the corresponding first allele are located, and

at which at least a second predetermined number of reads in the plurality of reads containing the corresponding second allele are located,

for each of the filtered informative fetal loci:

determining a first number of reads located at the filtered informative fetal locus and containing the corresponding first allele,

determining a second number of reads located at the filtered informative fetal locus and containing the corresponding second allele,

calculating a sum of the first number and the second number,

calculating a fetal ratio of the second number divided by the sum,

determining a scalar representing a total expression at the filtered informative fetal locus relative to expression of the corresponding second allele in the fetus;

multiplying the fetal ratio by the scalar to obtain a fetal contribution, and

determining a portion of RNA in the sample that is of fetal origin, wherein the portion is the average of the fetal contributions for the filtered informative maternal loci and the filtered informative fetal loci.

24. The method of claim 23, wherein the average is weighted by the sums for the filtered informative maternal loci and the filtered informative fetal loci.

25. The method of claim 23, wherein the scalar is assumed to be about 2 for a filtered informative fetal locus.

26. The method of claim 23, wherein the first predetermined number of reads for the one or more filtered informative fetal loci is 1 and the second predetermined number of reads for the one or more filtered informative fetal loci is 1.

27. The method of claim 23, further comprising sequencing genomic DNA obtained from maternal tissue to determine a genotype of the pregnant female subject at each informative fetal locus.

28. The method of claim 23, further comprising sequencing genomic DNA obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a genotype of the fetus at each informative fetal locus.

29. The method of claim 13 or 23, further comprising diagnosing a pregnancy-associated disorder by comparing the portion of RNA in the sample that is of fetal origin to a cutoff value.

30. A method of designating a genomic locus as a maternal or fetal marker by analyzing a sample from a female subject pregnant with a fetus, the method comprising:

receiving a plurality of reads, wherein the reads are obtained from an analysis of RNA molecules obtained from the sample, the sample containing a mixture of maternal- and fetal-derived RNA molecules;

identifying, by a computer system, locations of the reads in a reference sequence;

identifying one or more informative loci, each of which is homozygous in a first entity for a corresponding first allele and which is heterozygous in a second entity for the corresponding first allele and a corresponding second allele, wherein the first entity is the pregnant female subject or the fetus, and the second entity is the other one of the pregnant female subject and the fetus;

filtering the one or more informative loci to identify one or more filtered informative loci:

that are located within an expressed region of the reference sequence,

at which at least a first predetermined number of reads in the plurality of reads containing the corresponding first allele are located, and

at which at least a second predetermined number of reads in the plurality of reads containing the corresponding second allele are located,

for each of the filtered informative loci:

determining a first number of reads located at the filtered informative locus and containing the corresponding first allele,

determining a second number of reads located at the filtered informative locus and containing the corresponding second allele,

calculating a ratio of the first number and the second number,

designating the filtered informative locus as a marker for the second entity when the ratio exceeds a cutoff.

31. The method of claim 30, wherein the sample of the pregnant woman is a sample of blood plasma.

32. The method of claim 30, wherein the second entity is the pregnant female subject.
33. The method of claim 30, wherein the second entity is the fetus.
34. The method of claim 30, wherein calculating a ratio of the first number and the second number comprises dividing the second number the sum of the first number and the second number.
35. The method of claim 30, wherein the cutoff is from about 0.2 to about 0.5.
36. The method of claim 35, wherein the cutoff is 0.4.
37. The method of claim 30, wherein the analysis of RNA molecules obtained from the sample comprises sequencing the RNA molecules or cDNA copies thereof.
38. The method of claim 30, wherein the analysis of RNA molecules obtained from the sample comprises performing digital PCR.
39. The method of claim 30, wherein identifying locations of the reads in a reference sequence comprises aligning the reads to the reference sequence.
40. The method of claim 30, wherein the first predetermined number of reads is 1 and the second predetermined number of reads is 1.
41. The method of claim 30, further comprising sequencing genomic DNA obtained from maternal tissue to determine a genotype of the pregnant female subject at each informative locus.
42. The method of claim 30, further comprising sequencing genomic DNA obtained from the placenta, chorionic villi, amniotic fluid, or maternal plasma to determine a genotype of the fetus at each informative locus.
43. The method of claim 30, further comprising diagnosing a pregnancy-associated disorder based upon whether a filtered informative locus is designated as a marker for the second entity.

44. The method of claim 30, further comprising determining, for RNA in the sample that contains a filtered informative locus, a portion of the RNA that originates from the second entity, wherein the portion is determined by multiplying the ratio by a scalar.

45. The method of claim 44, wherein the scalar represents a total expression at the filtered informative maternal locus relative to expression of the corresponding second allele in the second entity.

46. The method of claim 44, wherein the scalar is assumed to be about 2.

47. The method of claim 44, further comprising determining a portion of the RNA that originates from the first entity by subtracting the portion of RNA that originates from the second entity from 1.

48. A system comprising one or more processors configured to perform the method of any one of claims 1 to 47.

49. A computer product comprising a computer readable medium storing a plurality of instructions for controlling a processor to perform the method of any one of claims 1 to 47.