

CLAIMS

1. A method of enriching fetal nuclei, the method comprising selecting fetal nuclei from a sample from a pregnant female.
2. The method of claim 1 which comprises selecting cellular material which is less than about 10 μ m in size.
3. The method of claim 1 which comprises selecting cellular material which is less than about 8 μ m in size.
4. The method according to any one of claims 1 to 3, wherein the cellular material is selected using: a cell strainer, flow cytometry, microfluidics, or a combination thereof.
5. The method of claim 1 which comprises selecting fetal nuclei using an agent which binds said nuclei.
6. The method of claim 5, wherein the agent binds a molecule selected from: a Nuclear Membrane Protein, a Nuclear Lamin, a Nuclear Pore Proteins and a transcription factor expressed by the fetal cells, or a combination thereof.
7. The method of claim 6, wherein the agent binds a molecule selected from: Nuclear Membrane Protein, Lamin A, Lamin B, Lamin C, Glial Cell Missing 1 (GCM1), Eomesodermin homolog protein (EOMES), Nucleoporin P62, Nuclear Envelope GP210, HoxB6, HoxC5, HoxC6, Hox3F, HB24, GAX, MSX2, DLX4, Pit-1, AP-2n, TEF-1, TEF-3, and Ets-1, or a combination thereof.
8. The method according to any one of claims 5 to 7, wherein the agent is an antibody or antibody fragment.
9. The method according to any one of claims 5 to 8, wherein the agent is bound to a detectable label or isolatable label.
10. The method according to any one of claims 5 to 8, wherein the method further comprises binding to the agent a detectable label or isolatable label.

11. The method of claim 9 or claim 10, wherein the label is selected from the group consisting of: a fluorescent label, a radioactive label, a paramagnetic particle, a chemiluminescent label, a label that is detectable by virtue of a secondary enzymatic reaction, and a label that is detectable by virtue of binding to a molecule.
12. The method according to any one of claims 9 to 11, wherein the step of selecting fetal nuclei comprises detecting the label and selecting the labelled nuclei.
13. The method of claim 12, wherein the detectable label or isolatable label is a fluorescent label and the step of selecting the fetal nuclei comprises performing fluorescence activated cell sorting.
14. The method of claim 12, wherein the detectable label or isolatable label is a paramagnetic particle and the step of selecting the fetal nuclei comprises exposing the labelled nuclei to a magnetic field.
15. The method according to any one of claims 1 to 14, wherein the sample is at least partially mechanically and/or enzymatically disaggregated before the fetal nuclei are selected.
16. The method according to any one of claims 1 to 15 which comprises selecting cellular material which is less than about 10 μ m and positively selecting fetal nuclei using an agent which binds said nuclei.
17. The method according to any one of claims 1 to 16 which further comprises selecting fetal cells.
18. The method of claim 17 which comprises
 - i) at least partially mechanically disaggregating the sample to produce a cellular material suspension,
 - ii) filtering the suspension through a first cell strainer which has a mesh size of at least about 100 μ m and collecting the cellular material that passed through the first cell strainer,
 - iii) filtering the cellular material collected in step ii) through a second cell strainer which has a mesh size of less than about 40 μ m and collecting the cellular

material that did not pass through the second cell strainer, and independently collecting the cellular material that passed through the second cell strainer,

iv) filtering the cellular material collected in step iii), which passed through the second cell strainer, through a third cell strainer which has a mesh size of less than about 10 μ m and collecting the cellular material that passed through the third cell strainer, and

v) combining the cellular material obtained from step iii), which did not pass through the second cell strainer, which comprises fetal cells with the cellular material obtained from step iv) which comprises fetal nuclei.

19. The method of claim 17 which comprises

i) at least partially enzymatically disaggregating the sample to produce a cellular material suspension,

ii) filtering the suspension through a first cell strainer which has a mesh size of less than about 40 μ m and collecting the cellular material that did not pass through the first cell strainer, and independently collecting the cellular material that passed through the first cell strainer,

iii) filtering the cellular material collected in step ii), which passed through the first cell strainer, through a second cell strainer which has a mesh size of less than about 10 μ m and collecting the cellular material that passed through the second cell strainer, and

iv) combining the cellular material obtained from step ii), which did not pass through the first cell strainer, which comprises fetal cells with the cellular material obtained from step iii) which comprises fetal nuclei.

20. The method of claim 17 which comprises

i) at least partially mechanically disaggregating the sample to produce a cellular material suspension,

ii) filtering the suspension through a cell strainer which has a mesh size of at least about 100 μ m and collecting the cellular material that passed through the cell strainer,

iii) sorting the cellular material collected in step ii) by fluorescent activated cell separation (FACS) based on forward scatter and collecting cellular material which is at least about 40 μ m in size,

iv) sorting the cellular material collected in step ii) and/or step iii) by fluorescent activated cell separation (FACS) based on forward scatter and collecting cellular material which is less than about 10 μ m, and

v) combining the cellular material obtained from step iii) which comprises fetal cells with the cellular material obtained from step iv) which comprises fetal nuclei.

21. The method of claim 17 which comprises

i) at least partially mechanically and/or enzymatically disaggregating the sample to produce a cellular material suspension,

ii) sorting the suspension by fluorescent activated cell separation (FACS) based on forward scatter and collecting cellular material which is between about 40 μ m and 100 μ m in size, and collecting cellular material which is less than about 10 μ m, and

iii) combining the cellular material which is between about 40 μ m and 100 μ m in size which comprises fetal cells, with the cellular material which is less than about 10 μ m comprising fetal nuclei.

22. The method of claim 17 which comprises positively selecting fetal cells using an agent which binds fetal cells but not maternal cells.

23. The method of claim 17 or claim 22 which comprises negatively selecting fetal cells using an agent which binds maternal cells but does not bind fetal cells.

24. The method of claim 23 or claim 24 which comprises combining the fetal nuclei and fetal cells.

25. The method according to any one of claims 15 to 24, wherein at least partially mechanically disaggregating the sample comprises gentle pipetting using an about 1ml pipette and/or using forceps.

26. The method according to any one of claims 15 to 25, wherein at least partially enzymatically disaggregating the sample comprises contacting the sample with a collagenase, a protease or a combination thereof.

27. A method of enriching fetal nuclei from a sample from a pregnant female, the method comprising

i) enriching fetal cells from the sample,

ii) contacting the fetal cells with an antibody that binds thereto, and
iii) inducing complement mediated lysis of the fetal cells bound to the antibody to release the fetal nuclei.

28. The method of claim 27, wherein the fetal cells are syncytiotrophoblasts and/or cytotrophoblasts.

29. The method of claim 27 or claim 28, wherein i) and ii) are conducted concurrently and the antibody is also used for the enrichment of the fetal cells.

30. The method according to any one of claims 27 to 29 which comprises
iv) further enriching the fetal nuclei using the method according to any one of claims 1 to 26.

31. The method according to any one of claims 1 to 30, wherein the sample is blood, cervical mucous or urine.

32. The method according to any one of claims 1 to 31, wherein the sample was obtained within 5 to 18 weeks of pregnancy.

33. The method according to any one of claims 1 to 32 which further comprising obtaining the sample.

34. An enriched population of fetal nuclei obtained by a method according to any one of claims 1 to 33.

35. A composition comprising fetal nuclei of claim 34, and a carrier.

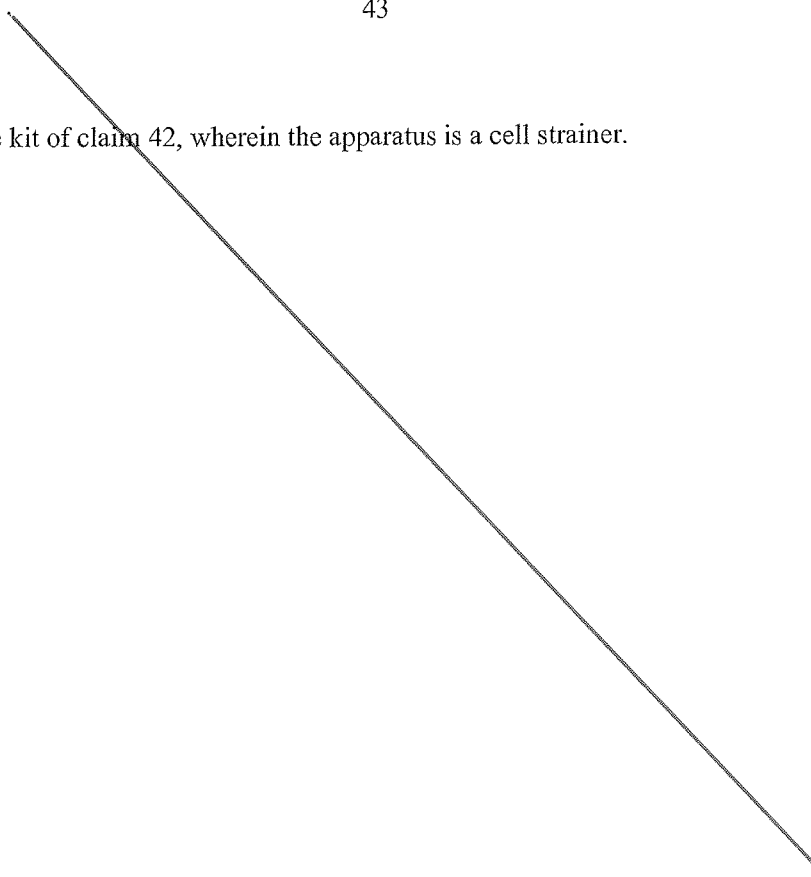
36. A method for analysing the genotype of a fetus at a locus of interest, the method comprising

i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 33, and

ii) analysing the genotype of at least one fetal nuclei at a locus of interest.

37. The method of claim 36, wherein the fetal nuclei is analysed for a genetic abnormality linked to a disease state, or predisposition thereto.

38. A method of determining the sex of a fetus, the method comprising
i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 33, and
ii) analysing at least one fetal nuclei to determine the sex of the fetus.
39. A method of determining the father of a fetus, the method comprising
i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 33,
ii) determining the genotype of the candidate father at one or more loci,
iii) determining the genotype of the fetus at one or more of said loci, and
iv) comparing the genotypes of ii) and iii) to determine the probability that the candidate father is the biological father of the fetus.
40. A kit for enriching fetal nuclei from a sample, the kit comprising at least two of the following;
i) an apparatus for obtaining the sample,
ii) an apparatus and/or media for transporting and/or storing the sample to a diagnostic laboratory,
iii) an apparatus for obtaining a second sample comprising maternal DNA but no fetal DNA from the mother,
iv) an apparatus for at least partially mechanically disaggregating the sample,
v) an enzyme for at least partially enzymatically disaggregating the sample,
vi) an antibody which binds fetal cells,
vii) a composition for performing complement mediated lysis,
ix) at least one apparatus or reagent for selecting fetal nuclei, and/or
x) a reagent(s) for performing a genetic assay.
41. The kit of claim 40 which comprises
i) an apparatus for at least partially mechanically disaggregating the sample, and
ii) at least one reagent for selecting fetal nuclei using magnetic separation.
42. The kit of claim 41 which comprises
i) an apparatus for at least partially enzymatically disaggregating the sample, and
and
ii) at least one apparatus for selecting fetal nuclei using size separation.

43. The kit of claim 42, wherein the apparatus is a cell strainer.
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CLAIMS

1. A method of enriching free fetal nuclei, the method comprising selecting fetal nuclei from a cervical mucous sample from a pregnant female by selecting cellular material which is less than 10 μ m in size.
2. The method of claim 1 which comprises selecting cellular material which is less than 8 μ m in size.
3. The method of claim 1 which further comprises selecting fetal nuclei using an agent which binds said nuclei, wherein the agent is an antibody or antibody fragment.
4. The method of claim 3, wherein the agent binds a Nuclear Membrane Protein, a Nuclear Lamin, a Nuclear Pore Protein or a Transcription Factor.
5. The method of claim 4, wherein the agent binds a molecule selected from: Nuclear Membrane Protein, Lamin A, Lamin B, Lamin C, Glial Cell Missing 1 (GCM1), Eomesodermin homolog protein (EOMES), Nucleoporin P62, and Nuclear Envelope GP210, HoxB6, HoxC5, HoxC6, Hox3F, HB24, GAX, MSX2, DLX4, Pit-1, AP-2n, TEF-1, TEF-3, and Ets-1, or a combination thereof.
6. The method according to any one of claims 1 to 5 which further comprises selecting fetal cells from said sample.
7. The method of claim 6 which comprises positively selecting fetal cells using an agent which binds fetal cells but not maternal cells.
8. The method of claim 6 which comprises negatively selecting fetal cells using an agent which binds maternal cells but does not bind fetal cells.
9. A method for analysing the genotype of a fetus at a locus of interest, the method comprising
 - i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 8, and
 - ii) analysing the genotype of at least one fetal nuclei at a locus of interest.
10. A method of determining the sex of a fetus, the method comprising

i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 8, and

ii) analysing at least one fetal nuclei to determine the sex of the fetus.

11. A method of determining the father of a fetus, the method comprising

i) obtaining enriched fetal nuclei using a method according to any one of claims 1 to 8,

ii) determining the genotype of the candidate father at one or more loci,

iii) determining the genotype of the fetus at one or more of said loci, and

iv) comparing the genotypes of ii) and iii) to determine the probability that the candidate father is the biological father of the fetus.