CLAIMS

What is claimed is:

1. A method for analyzing DNA from a single cell, said method comprising:

(a) performing whole genome amplification of the genome of a single cell to produce an amplified genome, wherein the method entails performing whole genome amplification for fewer than 10 amplification cycles;

(b) preamplifying the amplified genome to produce a preamplification reaction mixture comprising a plurality of amplicons specific for target nucleic acids, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification, and said chambers are from 2 nL to 500 nL in volume; and

(c) amplifying and detecting the plurality of amplicons, wherein said amplifying and detecting comprises carrying out at least 5 assays for amplicons of interest.

2. A method for analyzing mRNA from a single cell, said method comprising:

(a) preparing DNA from the mRNA of a single cell;

(b) preamplifying the DNA to produce a preamplification reaction mixture comprising a plurality of amplicons specific for target nucleic acids, wherein said preamplification is carried out for 18 cycles or fewer, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification, and said chambers are from 2 nL to 500 nL in volume; and

(c) amplifying and detecting the plurality of amplicons, wherein the amplicon(s) is/are detected by a method selected from a real-time quantitation method, monitoring the amount of amplification product after a predetermined number of cycles sufficient to indicate the presence of the target nucleic acids in the sample, and melting curve analysis, wherein said amplifying and detecting comprises carrying out at least 5 assays for amplicons of interest.

3. A method for analyzing non-coding RNA from a single cell, said method comprising:

(a) preparing DNA from the non-coding RNA of a single cell;

(b) preamplifying the DNA to produce a preamplification reaction mixture comprising a plurality of amplicons specific for target nucleic acids, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification, and said chambers are from 2 nL to 500 nL in volume; and

(c) amplifying and detecting the a plurality of amplicons by a method selected from a real-time quantitation method, monitoring the amount of amplification product after a predetermined number of cycles sufficient to indicate the presence of the target nucleic acids in the sample, and melting curve analysis, wherein said amplifying and detecting comprises carrying out at least 5 assays for amplicons of interest.

4. A method for analyzing DNA from a single cell, said method comprising;

(a) performing whole genome amplification of the genome of a single cell to produce an amplified genome;

(b) preamplifying the amplified genome to produce a preamplification reaction mixture comprising a plurality of amplicons specific for target nucleic acids, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification, and said chambers are from 2 nL to 500 nL in volume; and

(c) amplifying and detecting the a plurality of amplicons, wherein primer pairs used in preamplification and/or amplification can amplify and detect single nucleotide polymorphisms (SNPs) by a method selected from a real-time quantitation method, monitoring the amount of amplification product after a predetermined number of cycles sufficient to indicate the presence of the target nucleic acids in the sample, and melting curve analysis, wherein said amplifying and detecting comprises carrying out at least 5 assays for amplicons of interest.

5. A method for analyzing RNA from a single cell, said method comprising;

(a) preparing DNA from the RNA of a single cell;

(b) preamplifying the DNA to produce a preamplification reaction mixture comprising a plurality of amplicons specific for one of more target nucleic acids, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification, and said chambers are from 2 nL to 500 nL in volume; and

(c) amplifying and detecting the a plurality of amplicons, wherein primer pairs used in preamplification and/or amplification can amplify and detect single nucleotide polymorphisms (SNPs) by a method selected from a real-time quantitation method, monitoring the amount of amplification product after a predetermined number of cycles sufficient to indicate the presence of the target nucleic acids in the sample, and melting curve analysis, wherein said amplifying and detecting comprises carrying out at least 5 assays for amplicons of interest.

6. The method of any of claims 1-5, wherein said single cell comprises a mammalian cell.

7. The method of claim 6, wherein said single cell is selected from a cell from a preimplantation embryo, a stem cell, a suspected cancer cell, a cell from a pathogenic organism, and a cell obtained from a crime scene.

8. The method of claim 7, wherein the cell comprises a human blastomere.

9. The method of claim 8, wherein the human blastomere is from an eight-cell stage embryo.

10. The method of claim 7, wherein the cell comprises a human stem cell.

11. The method of claim 4, wherein said whole genome amplification is stopped before a reaction plateau is reached.

12. The method of claims 1 or 4, wherein said whole genome amplification is performed for more than two amplification cycles.

13. The method of claim 4, wherein said whole genome amplification is performed for fewer than 10 amplification cycles.

14. The method of claim 12, wherein said whole genome amplification is performed for between four and eight cycles, inclusive.

15. The method of claims 1 or 4, wherein said whole genome amplification is carried out using a technique selected from the group consisting of primer extension PCR (PEP), degenerated oligonucleotide primed PCR, ligation-mediated PCR (LMP), the T7-based linear amplification of DNA (TLAD), and multiple displacement amplification (MDA).

16. The method of claim 2, wherein the DNA is cDNA produced by reverse transcription of mRNA.

17. The method of claim 5, wherein the RNA comprises non-coding RNA.

18. The method of claims 17 or 3, wherein the non-coding RNA is selected from the group consisting of small nucleolar RNA (snoRNA), microRNA (miRNA), small interfering RNA (siRNA), and Piwi-interacting RNAs (piRNA).

19. The method of claims 17 or 3, wherein DNA is produced from the non-coding RNA by reverse transcription or amplification.

20. The method of any of the preceding claims, wherein said preamplification is carried out using primer pairs specific for said target nucleic acids.

21. The method of any of the preceding claims, wherein said preamplification is carried out for 8-18 cycles.

22. The method of any of the preceding claims, wherein said amplification of (c) is carried out using primer pairs specific for said target nucleic acids.

23. The method of claims 16 or 19, wherein DNA is produced by reverse transcription, which is followed by preamplification in the same reaction mixture.

24. The method of any of claims 16, 19, or 23 wherein no probe is present in the preamplification mixture.

25. The method of claim 20, wherein said primer pairs amplify single nucleotide polymorphisms (SNPs).

26. The method of claim 25, wherein the presence of one or more of said SNPs is/are correlated with the presence of one or more genetic defects.

27. The method of claims 1-5, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

28. The method of any of claims 1-5, wherein the preamplification and/or the amplification is carried out by polymerase chain reaction (PCR).

29. The method of any of claims 1-5, wherein the presence of an amplification product is determined by quantitative real-time polymerase chain reaction (qPCR).

30. The method of claim 29, wherein a universal qPCR probe is employed in the amplification mixtures to detect amplification products.

31. The method of claim 30, wherein the universal qPCR probe comprises a double-stranded DNA (dsDNA) dye.

32. The method of claim 29, wherein one or more target-specific qPCR probes is employed in the amplification mixtures to detect amplification products.

33. The method of claim 29, wherein the presence of an amplification product is detected using a fluorogenic nuclease assay.

34. The assay method of claim 33, wherein the presence of an amplification product is detected using a dual-labeled fluorogenic oligonucleotide probe.

35. The method of any of claims 1-5, wherein the plurality of amplicons comprises at least 10 amplicons.

36. The method of any of claims 1-5, wherein the plurality of amplicons comprises from 5 to 96 amplicons.

37. The method of claims 1-5, wherein different amplicons are amplified and detected in different chambers.

38. The method of any preceding claim, wherein said amplifying and detecting is performed in a low-volume PCR reaction apparatus comprising reaction chambers having a volume of from about 2 nL to about 500 nL.

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CLAIMS

1. A method for analyzing DNA from a single cell, said method comprising:

(a) performing whole genome amplification of the genome of a single cell to produce an amplified genome, wherein the method entails performing whole genome amplification for fewer than, or about, 10 amplification cycles;

(b) preamplifying the amplified genome using a plurality of target-specific primer pairs to produce a preamplification reaction mixture comprising a plurality of amplicons specific for a plurality of target nucleic acids;

(c) distributing the plurality of amplicons within a device; and

(d) amplifying and detecting the plurality of amplicons in the device.

2. A method for analyzing mRNA from a single cell, said method comprising:

(a) preparing DNA from the mRNA of a single cell;

(b) preamplifying the DNA using a plurality of target-specific primer pairs to produce a preamplification reaction mixture comprising a plurality of amplicons specific for a plurality of target nucleic acids, wherein said preamplification is carried out for 18 amplification cycles or fewer;

(c) distributing the plurality of amplicons within a device; and

(d) amplifying and detecting the plurality of amplicons in the device.

3. The method of any of claims 1, 2, or 31-33, wherein said single cell comprises a mammalian cell.

4. The method of claim 3, wherein said single cell is selected from a cell from a preimplantation embryo, a stem cell, a suspected cancer cell, a cell from a pathogenic organism, and a cell obtained from a crime scene.

5. The method of claim 4, wherein the cell comprises a human blastomere.

6. The method of claim 5, wherein the human blastomere is from an eight-cell stage embryo.

7. The method of claim 4, wherein the cell comprises a human stem cell.

8. The method of claim 32, wherein said whole genome amplification is carried out such that a reaction plateau is not reached.

9. The method of claims 1 or 32, wherein said whole genome amplification is performed for more than two amplification cycles.

10. The method of claim 32, wherein said whole genome amplification is performed for fewer than, or about, 10 amplification cycles.

11. The method of claim 9, wherein said whole genome amplification is performed for between four and eight cycles, inclusive.

12. The method of claims 1 or 32, wherein said whole genome amplification is carried out using a technique selected from the group consisting of primer extension PCR (PEP), degenerated oligonucleotide primed PCR, ligation-mediated PCR (LMP), the T7-based linear amplification of DNA (TLAD), and multiple displacement amplification (MDA).

13. The method of claim 2, wherein the DNA is cDNA produced by reverse transcription of mRNA.

14. The method of claim 33, wherein the RNA comprises non-coding RNA.

15. The method of claims 14 or 31, wherein the non-coding RNA is selected from the group consisting of small nucleolar RNA (snoRNA), microRNA (miRNA), small interfering RNA (siRNA), and Piwi-interacting RNAs (piRNA).

16. The method of claims 14 or 31, wherein DNA is produced from the non-coding RNA by reverse transcription or amplification.

17. The method of claims 1 or 33, wherein said preamplification is carried out for 8-18 cycles.

18. The method of any of claims 1, 2, or 31-33, wherein said amplification is carried out using one or more primer pairs specific for said one or more target nucleic acids.

19. The method of claim 13, wherein DNA is produced by reverse transcription, which is followed by preamplification in the same reaction mixture.

20. The method of claim 13, wherein no probe is present in the preamplification mixture.

21. The method of claims 1, 2, or 31-33, wherein said plurality of target-specific primer pairs used for preamplification amplifies one or more single nucleotide polymorphisms (SNPs).

22. The method of claim 21, wherein the presence of said one or more SNPs is/are correlated with the presence of one or more genetic defects.

23. The method of any of claims 1, 2, or 31-33, wherein the device comprises a microfluidic device is fabricated, at least in part, from an elastomeric material.

24. The method of any of claims 1, 2, or 31-33, wherein the preamplification and the amplification is carried out by polymerase chain reaction (PCR).

25. The method of any of claims 1, 2, or 31-33, wherein the presence of an amplification product is determined by quantitative real-time polymerase chain reaction (qPCR).

26. The method of claim 25, wherein a universal qPCR probe is employed in the amplification mixtures to detect amplification products.

27. The method of claim 26, wherein the universal qPCR probe comprises a double-stranded DNA (dsDNA) dye.

28. The method of claim 25, wherein one or more target-specific qPCR probes is employed in the amplification mixtures to detect amplification products.

29. The method of claim 25, wherein the presence of an amplification product is detected using a fluorogenic nuclease assay.

30. The assay method of claim 29, wherein the presence of an amplification product is detected using a dual-labeled fluorogenic oligonucleotide probe.

31. A method for analyzing non-coding RNA from a single cell, said method comprising:

(a) preparing DNA from the non-coding RNA of a single cell;

(b) preamplifying the DNA using a plurality of target-specific primer pairs to produce a preamplification reaction mixture comprising a plurality of amplicons specific for one or more target nucleic acids;

(c) distributing the plurality of amplicons within a device; and

(d) amplifying and detecting the plurality of amplicons in the device.

32. A method for analyzing DNA from a single cell, said method comprising:

(a) performing whole genome amplification of the genome of a single cell to produce an amplified genome, wherein the whole genome amplification comprises polymerase chain reaction (PCR) cycles;

(b) preamplifying the amplified genome using a plurality of target-specific primer pairs to produce a preamplification reaction mixture comprising a plurality of amplicons specific for one or more target nucleic acids;

(c) distributing the plurality of amplicons within a device; and

(d) amplifying and detecting the plurality of amplicons in the device.

33. A method for analyzing RNA from a single cell, said method comprising:

(a) preparing DNA from the RNA of a single cell;

(b) preamplifying the DNA using a plurality of target-specific primer pairs to produce a preamplification reaction mixture comprising a plurality of amplicons specific for one or more target nucleic acids;

(c) distributing the plurality of amplicons within a device; and

(d) amplifying and detecting the plurality of amplicons in the device.

34. The method of any of claims 1, 2, or 31-33, wherein the plurality of amplicons comprises at least 10 amplicons.

35. The method of any of claims 1, 2, or 31-33, wherein the plurality of amplicons comprises from 10 to 96 amplicons.