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 ~~one or 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 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 ~~one or more~~ 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.

63. The method of any of claims 1-5, ~~2, or 33, 34, or 35~~, wherein said single cell comprises a mammalian cell.

74. The method of claim 63, 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.

85. The method of claim 74, wherein the cell comprises a human blastomere.

96. The method of claim 85, wherein the human blastomere is from an eight-cell stage embryo.

107. The method of claim 74, wherein the cell comprises a human stem cell.

118. The method of claim 434, wherein said whole genome amplification is stopped before a reaction plateau is reached.

129. The method of claims 1 or 434, wherein said whole genome amplification is performed for more than two amplification cycles.

1310. The method of claim 434, wherein said whole genome amplification is performed for fewer than 10 amplification cycles.

1411. The method of claim 129, wherein said whole genome amplification is performed for between four and eight cycles, inclusive.

1512. The method of claims 1 or 434, 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).

1613. The method of claim 2, wherein the DNA is cDNA produced by reverse transcription of mRNA.

1714. The method of claim 535, wherein the RNA comprises non-coding RNA.

1815. The method of claims 1714 or 333, 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).

1916. The method of claims 1714 or 333, wherein DNA is produced from the non-coding RNA by reverse transcription or amplification.

2017. The method of any of the preceding claims ~~or of claims 33, 34, or 35~~, wherein said preamplification is carried out using ~~one or more~~ primer pairs specific for said ~~one or more~~ target nucleic acids.

2118. The method of any of the preceding claims ~~or of claims 33, 34, or 35~~, wherein said preamplification is carried out for 8-18 cycles.

2219. The method of any of the preceding claims ~~or of claims 33, 34, or 35~~, wherein said amplification of (c) is carried out using ~~one or more~~ primer pairs specific for said ~~one or more~~ target nucleic acids.

2320. The method of claims 1613 or 1916, wherein DNA is produced by reverse transcription, which is followed by preamplification in the same reaction mixture.

2421. The method of any of claims 1613, 1916, or 2320 wherein no probe is present in the preamplification mixture.

2522. The method of claim 2017, wherein said ~~one or more~~ primer pairs amplify~~used for preamplification amplifies one or more~~ single nucleotide polymorphisms (SNPs).

2623. The method of claim 2522, wherein the presence of ~~said~~ one or more of said SNPs is/are correlated with the presence of one or more genetic defects.

~~24. The method of any of claims 1, 2, or claims 33, 34, or 35, wherein the preamplification mixture is distributed into separate chambers of a microfluidic device prior to amplification.~~

2725. The method of claims 1-524, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

2826. The method of any of claims 1-5, ~~2, or claims 33, 34, or 35,~~ wherein the preamplification and/or the amplification is carried out by polymerase chain reaction (PCR).

2927. The method of any of claims 1-5, ~~2, or claims 33, 34, or 35,~~ wherein the presence of an amplification product is determined by quantitative real-time polymerase chain reaction (qPCR).

3028. The method of claim 2927, wherein a universal qPCR probe is employed in the amplification mixtures to detect amplification products.

3129. The method of claim 3028, wherein the universal qPCR probe comprises a double-stranded DNA (dsDNA) dye.

3230. The method of claim 2927, wherein one or more target-specific qPCR probes is employed in the amplification mixtures to detect amplification products.

3331. The method of claim 2927, wherein the presence of an amplification product is detected using a fluorogenic nuclease assay.

3432. The assay method of claim 3331, wherein the presence of an amplification product is detected using a dual-labeled fluorogenic oligonucleotide probe.

~~33. 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 one or more target nucleic acids; 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, and melting curve analysis.~~

~~34. 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 one or more target nucleic acids; 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, and melting curve analysis.~~

~~35. 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 or more target nucleic acids; 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, and melting curve analysis.~~

35~~36~~. The method of any of claims 1-5, ~~2, or claims 33, 34, or 35,~~ wherein the plurality of amplicons comprises at least 10 amplicons.

3637. The method of any of claims 1-5, ~~2, or claims 33, 34, or 35,~~ wherein the plurality of amplicons comprises from 510 to 96 amplicons.

3738. The method of claims 1-5 ~~24~~, 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.