

CLAIMS

1. A method for amplifying a plurality of target nucleic acids in a plurality of samples, the method comprising:
 - 5 preparing an amplification mixture for each target nucleic acid in a microfluidic device, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material and comprises:
 - a plurality of first input lines, each comprising a first input port at one end;
 - 10 a plurality of second input lines, each comprising a second input port at one end;
 - a plurality of harvesting reagent input lines, each in fluid communication with another end of each second input line, wherein each harvesting reagent input line comprises a
 - 15 harvesting reagent input port;
 - a plurality of sets of first chambers, wherein each set of first chambers is in fluid communication with one of the plurality of first input lines;
 - a plurality of sets of second chambers, wherein each set of
 - 20 second chambers is in fluid communication with one of the plurality of second input lines and one of the plurality of harvesting reagent input lines;
 - a plurality of first set-adjacent valves for dilation pumping, each disposed in one of the plurality of second input lines
 - 25 between the second input port and the set of second chambers; and
 - a plurality of second set-adjacent valves for dilation pumping, each disposed in one of the plurality of harvesting reagent input lines;
 - 30 and the microfluidic device permits recovery of pools of reaction products by dilation pumping, and the variability, with respect to volume or copy number, of each pool

recovered from the device is less than 10 percent, each amplification mixture comprising:

a forward primer comprising a target-specific sequence;

and

5 a reverse primer comprising a target-specific sequence;

subjecting each amplification mixture to amplification to produce a plurality of target nucleotide sequences;

10 tagging the target nucleotide sequences to produce a plurality of target amplicons, each comprising first or second nucleotide tags flanking the target nucleotide sequence;

wherein the target amplicons comprise a DNA sequencing library, and performing automated sequencing of the plurality of target amplicons yields sequencing data wherein at least 50 percent of the target amplicons are present at greater than 50 percent of the average number of copies of target amplicons and
15 less than 2-fold the average number of copies of target amplicons.

2. The method of claim 1 wherein at least 70 percent of the target amplicons are present at greater than 50 percent of the average number of copies of target amplicons and less than 2-fold the average number of copies of target amplicons.

20 3. The method of claim 1 or 2, wherein the average length of the target amplicons is at least 25 bases.

4. The method of claim 1 or 2, wherein the average length of the target amplicons is at least 50 bases.

25 5. The method of claim 1 or 2, wherein the average length of the target amplicons is at least 100 bases.

6. The method of claim 1 or 2, wherein the average length of the target amplicons is at least 200 bases.

7. The method of claim 1 or 2, wherein the average length of the target amplicons is at least 1 kilobase.

8. The method of claim 1, wherein at least 90 percent of the target amplicons are present at greater than 50 percent of the average number of copies of target amplicons and less than 2-fold the average number of copies of target amplicons.
- 5 9. The method of claim 1, wherein the volume of the amplification mixtures is in the range of 1 picoliter to 50 nanoliters.
10. The method of claim 1, wherein the volume of the amplification mixtures is in the range of 5 picoliters to 25 nanoliters.
- 10 11. The method of claim 1, wherein the amplification is carried out by polymerase chain reaction (PCR).
12. The method of claim 1, 2, or 8, additionally comprising recovering the target amplicons from the amplification mixtures.
- 15 13. The method of claim 12, additionally comprising subjecting at least one target amplicon to amplification using primers specific for the first and second nucleotide tags to produce a target amplicon lacking the barcode nucleotide sequence.
14. The method of claim 1, wherein the target nucleic acids comprise genomic DNA.
- 20 15. The method of claim 1, wherein the first and second nucleotide tags are capable of being bound by DNA sequencing primers.
16. The method of claim 15, additionally comprising subjecting at least one target amplicon to DNA sequencing.
17. The method of claim 1, additionally comprising quantifying the amount of target amplicons in the amplification mixtures.
- 25 18. The method of claim 17, wherein said quantifying comprises recovering the target amplicons and subjecting them to digital amplification.

19. The method of claim 18, wherein said digital amplification comprises:
- distributing the preamplified target amplicons into discrete reaction mixtures, wherein each reaction mixture, on average, includes no more than one amplicon per reaction mixture; and
- subjecting the reaction mixtures to amplification.
20. The method of claim 19, wherein said digital amplification comprises real-time PCR.
21. The method of claim 19, wherein said digital amplification comprises endpoint PCR.
22. The method of claim 19, wherein the amplification mixtures are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification.
23. The method of claim 1, wherein the presence of a target amplicon is determined by quantitative real-time polymerase chain reaction (qPCR).
24. The method of claim 1, wherein a universal qPCR probe is employed in the amplification mixtures to detect target amplicons.
25. The method of claim 1, wherein one or more target-specific qPCR probes is employed in the amplification mixtures to detect target amplicons.
26. The method of claim 1, wherein the presence of a target amplicon is detected using a fluorogenic nuclease assay.
27. The method of claim 1, wherein the presence of a target amplicon is detected using a dual-labeled fluorogenic hydrolysis oligonucleotide probe.
28. The method of claim 1, additionally comprising determining the amount of each target nucleic acid present in each sample.
29. The method of claim 1, wherein the method is performed in determining the copy numbers of the target nucleic acids in each sample.

30. The method of claim 1, wherein the method is performed in determining the genotypes at loci corresponding to the target nucleic acids.

31. The method of claim 1, wherein the method is performed in determining the expression levels of the target nucleic acids.

5 32. The method of claim 1, 2, or 8, wherein the method is performed to prepare target nucleic acids for sequencing.