Claims

1. A method for amplifying a plurality of target nucleic acids in a plurality of samples, the method comprising:

preparing an amplification mixture for each target nucleic acid, said amplification mixture comprising:

a forward primer comprising a target-specific portion;

a reverse primer comprising a target-specific portion, wherein the forward primer additionally comprises a first nucleotide tag and/or the reverse primer additionally comprises a second nucleotide tag; and

at least one barcode primer comprising a barcode nucleotide sequence and a first and/or second nucleotide tag-specific portion, wherein the concentration of the barcode primer is at least 5 fold the concentration of the forward and/or reverse primer(s);

subjecting each amplification mixture to amplification to produce a plurality of target amplicons comprising tagged target nucleotide sequences, each comprising first and/or second nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon.

2. The method of claim 1, 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 less than 2-fold the average number of copies of target amplicons.

3. The method of claim 2, wherein at least 70 percent, preferably at least 90%, 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.

4. The method of claim 2 or claim 3, wherein the target amplicons comprise a DNA sequencing library.

5. The method of any preceding claim, wherein the forward primer additionally comprises a first nucleotide tag; and/or the reverse primer additionally comprises a second nucleotide tag; and/or

the concentration of the barcode primer in the amplification mixtures is at least 50-fold the concentration of the forward and/or reverse primer(s); and/or

the first and/or second nucleotide tags and/or the barcode nucleotide sequence are selected so as to avoid substantial annealing to the target nucleic acids; and/or

the barcode nucleotide sequence identifies a particular sample; and/or

wherein the barcode primer comprises a barcode nucleotide sequence and a first nucleotide tag-specific portion, optionally wherein a plurality of forward primers comprise the same first nucleotide tag, preferably all forward primers used to amplify target sequences in each sample comprise the same first nucleotide tag; and/or

the amplification is carried out for at least 3 cycles to introduce the first and second nucleotide tags and the barcode nucleotide sequence, preferably the amplification is carried out for between 5 and 50 cycles, more preferably the amplification is carried out for a sufficient number of cycles to normalize target amplicon copy number across targets and across samples and/or

wherein one or more of the forward primer, reverse primer, and barcode primer comprises at least one additional primer binding site, preferably the barcode primer comprises at least a first additional primer binding site upstream of the barcode nucleotide sequence, which is upstream of the first nucleotide tag, more preferably the reverse primer comprises at least a second additional primer binding site downstream of the second nucleotide tag, preferably the first and second additional primer binding sites are capable of being bound by DNA sequencing primers; and/ or

subjecting at least one target amplicon to DNA sequencing.

6. The method of any preceding claim, wherein:

the forward primer comprises an inner forward primer comprising said target-specific portion and said first nucleotide tag;

the reverse primer comprises an inner reverse primer comprising said target-specific portion and said second nucleotide tag; and

the amplification mixture comprises two outer primers, wherein one of the outer primers is said barcode primer, wherein:

a first outer primer comprises said first nucleotide tag-specific portion; and

a second outer primer comprises said second nucleotide tag-specific portion, wherein one or both outer primer(s) comprise(s) said barcode nucleotide sequence, wherein the concentration of the outer primers is at least 5 fold the concentration of the inner primers.

7. The method of claim 6, wherein:

the first outer primer comprises a first additional primer binding site upstream of the barcode nucleotide sequence, which is upstream of the first nucleotide tag-specific portion; and

the second outer primer comprises a second tag-specific portion, an additional barcode nucleotide sequence, and downstream thereof, a second additional primer binding site.

8. The method of claim 5, wherein the forward and reverse primers for each target are initially combined separately from the sample, and each barcode primer is initially combined with its corresponding sample, preferably T targets are amplified in S samples, T and S being integers greater than one, the method additionally comprising preparing SXT amplification mixtures wherein the initially combined forward and reverse primers are added to the initially combined samples and barcode primers.

9. The method of any preceding claim where the average length of the target amplicons is at least 25 bases, optionally at least 50 bases, optionally at least 100 bases, optionally at least 200 bases, optionally at least 1 kilobase.

10. The method of any preceding claim, wherein the volume of the amplification mixtures is in the range of about 1 picoliter to about 50 nanoliters; preferably when dependent on claim 5 the volume of the amplification mixtures is in the range of about 5 picoliters to about 25 nanoliters.

11. The method of any preceding claim, wherein the amplification is carried out by polymerase chain reaction (PCR); said method optionally 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; and/or

wherein the target nucleic acids comprise genomic DNA; and/or

a) the method additionally comprising quantifying the amount of target amplicons in the amplification mixtures, preferably the quantifying comprises recovering the target amplicons and subjecting them to digital amplification,

preferably 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;

wherein said digital amplification optionally comprises real-time PCR; or endpoint PCR; or

b) wherein the presence of a target amplicon is determined by quantitative real-time polymerase chain reaction (qPCR), optionally employing a universal qPCR probe or one or more target-specific qPCR probes; or

c) wherein the presence of a target amplicon is detected using a fluorogenic nuclease assay, optionally using a dual-labeled fluorogenic hydrolysis oligonucleotide probe; and/or

optionally comprising determining the amount of each target nucleic acid present in each sample and/or determining the copy numbers of the target nucleic acids in each sample and/or determining the genotypes at loci corresponding to the target nucleic acids and/or determining the expression levels of the target nucleic acids; and/or

wherein the method is performed to prepare target nucleic acids for sequencing.

12. The method of claim 1 or 11, wherein the amplification mixtures are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification and optionally the microfluidic device is fabricated, at least in part, from an elastomeric material.

13. The method of any preceding claim, wherein the target amplicons are recovered from the amplification mixtures.

14. The method of claim 12, wherein, the target amplicons are recovered in a volume and/or copy number that varies less than about 50% among the recovered target amplicons.

CLAIMS

What is claimed is:

1. A method for amplifying a plurality of target nucleic acids in a plurality of samples, the method comprising:

preparing an amplification mixture for each target nucleic acid, said amplification mixture comprising:

a forward primer comprising a target-specific portion;

a reverse primer comprising a target-specific portion, wherein the forward primer additionally comprises a first nucleotide tag and/or the reverse primer additionally comprises a second nucleotide tag; and

at least one barcode primer comprising a barcode nucleotide sequence and a first and/or second nucleotide tag-specific portion, wherein the barcode primer is in excess of the forward and/or reverse primer(s);

subjecting each amplification mixture to amplification to produce a plurality of target amplicons comprising tagged target nucleotide sequences, each comprising first and/or second nucleotide tags flanking the target nucleotide sequence, and at least one barcode nucleotide sequence at the 5' or 3' end of the target amplicon.

2. The method of claim 1, wherein the forward primer additionally comprises a first nucleotide tag.

3. The method of claims 1 or 2, wherein the reverse primer additionally comprises a second nucleotide tag.

4. The method of claim 1, wherein the concentration of the barcode primer in the amplification mixtures is at least 4-fold the concentration of the forward and/or reverse primer(s).

5. The method of claim 4, wherein the concentration of the barcode primer in the amplification mixtures is at least 50-fold the concentration of the forward and/or reverse primer(s).

6. The method of claim 1, wherein the first and/or second nucleotide tags and/or the barcode nucleotide sequence are selected so as to avoid substantial annealing to the target nucleic acids.

7. The method of claim 1, wherein the barcode nucleotide sequence identifies a particular sample.

8. The method of claims 1 or 7, wherein the barcode primer comprises a barcode nucleotide sequence and a first nucleotide tag-specific portion.

9. The method of claim 8, wherein a plurality of forward primers comprise the same first nucleotide tag.

10. The method of claim 9, wherein the all forward primers used to amplify target sequences in each sample comprise the same first nucleotide tag.

11. The method of claim 7, wherein the forward and reverse primers for each target are initially combined separately from the sample, and each barcode primer is initially combined with its corresponding sample.

12. The method of claim 11, wherein T targets are amplified in S samples, T and S being integers greater than one, the method additionally comprising preparing S×T amplification mixtures wherein the initially combined forward and reverse primers are added to the initially combined samples and barcode primers.

13. The method of claim 1, wherein the amplification is carried out for at least 3 cycles to introduce the first and second nucleotide tags and the barcode nucleotide sequence.

14. The method of claim 13, wherein the amplification is carried out for between 5 and 50 cycles.

15. The method of claims 1, wherein the amplification is carried out for a sufficient number of cycles to normalize target amplicon copy number across targets and across samples.

16. The method of claim 1, 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 less than 2-fold the average number of copies of target amplicons.

17. A method for amplifying a plurality of target nucleic acids in a plurality of samples, the method comprising:

preparing an amplification mixture for each target nucleic acid, said amplification mixture comprising:

a forward primer comprising a target-specific sequence; and

a reverse primer comprising a target-specific sequence;

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

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

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 less than 2-fold the average number of copies of target amplicons.

18. The method of claim 16 or 17, 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.

19. The method of claims 16, 17, or 18, wherein the average length of the target amplicons is at least 25 bases.

20. The method of claims 16, 17, or 18, wherein the average length of the target amplicons is at least 50 bases.

21. The method of claims 16, 17, or 18, wherein the average length of the target amplicons is at least 100 bases.

22. The method of claims 16, 17, or 18, wherein the average length of the target amplicons is at least 200 bases.

23. The method of claims 16, 17, or 18, wherein the average length of the target amplicons is at least 1 kilobase.

24. The method of claim 16 or 17, 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.

25. The method of any of the preceding claims, wherein the volume of the amplification mixtures is in the range of about 1 picoliter to about 50 nanoliters.

26. The method of claim 17, wherein the volume of the amplification mixtures is in the range of about 5 picoliters to about 25 nanoliters.

27. The method of claims 1 or 17, wherein the amplification mixtures are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification.

28. The method of claim 27, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

29. The method of claims 1 or 17, wherein the amplification is carried out by polymerase chain reaction (PCR).

30. The method of claim 1 or 17, additionally comprising recovering the target amplicons from the amplification mixtures.

31. The method of claim 30, wherein the target amplicons are recovered in a volume and/or copy number that varies less than about 50% among the recovered target amplicons.

32. The method of claim 30, 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.

33. The method of claims 1 or 17, wherein the target nucleic acids comprise genomic DNA.

34. The method of claim 1, wherein one or more of the forward primer, reverse primer, and barcode primer comprises at least one additional primer binding site.

35. The method of claim 34, wherein the barcode primer comprises at least a first additional primer binding site upstream of the barcode nucleotide sequence, which is upstream of the first nucleotide tag.

36. The method of claim 35, wherein the reverse primer comprises at least a second additional primer binding site downstream of the second nucleotide tag.

37. The method of claim 36, wherein the first and second additional primer binding sites are capable of being bound by DNA sequencing primers.

38. The method of claim 17, wherein the first and second nucleotide tags are capable of being bound by DNA sequencing primers.

39. The method of claim 37 or 38, additionally comprising subjecting at least one target amplicon to DNA sequencing.

40. The method of claims 1 or 17, additionally comprising quantifying the amount of target amplicons in the amplification mixtures.

41. The method of claim 40, wherein said quantifying comprises recovering the target amplicons and subjecting them to digital amplification.

42. The method of claim 41, 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.

43. The method of claim 42, wherein said digital amplification comprises real-time PCR.

44. The method of claim 42, wherein said digital amplification comprises endpoint PCR.

45. The method of claim 42, wherein the amplification mixtures are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification.

46. The method of claim 45, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

47. The method of claims 1 or 17, wherein the presence of a target amplicon is determined by quantitative real-time polymerase chain reaction (qPCR).

48. The method of claims 1 or 17, wherein a universal qPCR probe is employed in the amplification mixtures to detect target amplicons.

49. The method of claims 1 or 17, wherein one or more target-specific qPCR probes is employed in the amplification mixtures to detect target amplicons.

50. The method of claims 1 or 17, wherein the presence of a target amplicon is detected using a fluorogenic nuclease assay.

51. The method of claims 1 or 17, wherein the presence of a target amplicon is detected using a dual-labeled fluorogenic hydrolysis oligonucleotide probe.

52. The method of claims 1 or 17, additionally comprising determining the amount of each target nucleic acid present in each sample.

53. The method of claims 1 or 17, wherein the method is performed in determining the copy numbers of the target nucleic acids in each sample.

54. The method of claims 1 or 17, wherein the method is performed in determining the genotypes at loci corresponding to the target nucleic acids.

55. The method of claims 1 or 17, wherein the method is performed in determining the expression levels of the target nucleic acids.

56. The method of claims 1 or 17, wherein the method is performed to prepare target nucleic acids for sequencing.

57. A microfluidic device comprising:

a plurality of first input lines;

a plurality of second input lines;

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 second chambers is in fluid communication with one of the plurality of second input lines;

a plurality of first pump elements in fluid communication with a first portion of the plurality of second input lines; and

a plurality of second pump elements in fluid communication with a second portion of the plurality of second input lines.

58. The microfluidic device of claim 57 wherein the first chambers comprise assay chambers and the second chambers comprise sample chambers.

59. The microfluidic device of claim 57 further comprising a set of valves preventing fluid communication between sample chambers making up a set of sample chambers.

60. The microfluidic device of claim 59 further comprising a set of valves preventing fluid communication between assay chambers making up a set of assay chambers and a sample chamber from each of the sets of sample chambers.

61. The microfluidic device of claim 57 wherein the plurality of first pump elements comprise a plurality of first valves.

62. The microfluidic device of claim 57 wherein the plurality of second pump elements comprise a plurality of second valves.

63. The microfluidic device of claim 57 wherein the microfluidic device comprises elastomeric material.

64. The microfluidic device of claim 57 wherein for a set of the plurality of sets of first chambers:

one of the first chambers is in fluid communication with a second chamber of a first set of second chambers via a fluid line;

another of the first chambers is in fluid communication with a second chamber of a second set of second chambers via another fluid line;

a first valve is operable to obstruct fluid flow through the fluid line; and

a second valve is operable to obstruct fluid flow through the another fluid line.

65. The microfluidic device of claim 64 wherein the first valve and the second valve are actuated using a common source.

66. A method of operating a microfluidic device having an assay chamber, a sample chamber, and a harvesting port, the method comprising:

closing a fluid line between the assay chamber and the sample chamber;

flowing a sample into the sample chamber via a sample input line;

flowing an assay into the assay chamber via an assay input line;

opening the fluid line between the assay chamber and the sample chamber;

combining at least a portion of the sample and at least a portion of the assay to form a mixture;

reacting the mixture to form a reaction product;

closing the fluid line between the assay chamber and the sample chamber;

flowing a harvesting reagent from the harvesting port to the sample chamber; and

removing the reaction product from the microfluidic device.

67. The method of claim 66 further comprising thermocycling the mixture.

68. The method of claim 66 wherein removing the reaction product from the microfluidic device comprises flowing the reaction product through at least a portion of the sample input line to a sample input port.

69. The method of claim 68 wherein flowing the reaction product through at least a portion of the sample input line comprises performing dilation pumping.

70. The method of claim 69 wherein dilation pumping comprises a fluid flow rate of less than or equal to 10 µl per hour.

71. The method of claim 69 wherein performing dilation pumping comprises:

a) closing a first valve disposed between the sample chamber and the sample input port;

b) opening a second valve disposed between the harvesting port and the sample chamber;

c) closing the second valve;

d) opening the first valve; and

repeating steps (a) through (d) a predetermined number of times.

72. The method of claim 70 wherein the fluid flow rate is less than or equal to 5 µl per hour.

73. The method of claim 72 wherein the fluid flow rate is less than or equal to 1 µl per hour.

74. The method of claim 66 wherein combining at least a portion of the sample and at least a portion of the assay to form a mixture comprises a free interface diffusion process.

75. The method of claim 66 wherein removing the reaction product comprises a fluid flow rate of less than or equal to 10 microliters per hour.

76. The method of claim 75 wherein the fluid flow rate is less than or equal to 5 microliters per hour.

77. The method of claim 76 wherein the fluid flow rate is less than or equal to 2 microliters per hour.

78. The method of claim 77 wherein the fluid flow rate is less than or equal to 1 microliters per hour.

79. The method of claim 66 wherein removing the reaction product comprises removing at least 95% of the reaction product from the microfluidic device.

80. The method of claim 66 wherein flowing the sample into the sample chamber via the sample input line comprises applying pressure to a sample input port to pressurize the sample input line.

81. The method of claim 66 wherein flowing the assay into the assay chamber via the assay input line comprises applying pressure to an assay input port to pressurize the assay input line.

82. A method of preparing reaction products, the method comprising:

providing M samples;

providing N assays;

mixing the M samples and N assays to form MxN pairwise combinations, each of the MxN pairwise combinations being contained in a closed volume;

forming MxN reaction products from the MxN pairwise combinations; and

recovering the MxN reaction products.

83. The method of claim 82 wherein the M samples are contained in M sets of first chambers and the N assays are contained in N sets of second chambers.

84. The method of claim 83 wherein the first chambers containing one of the M samples are associated with a set of second chambers, each of the set of second chambers containing one of the N assays.

85. The method of claim 84 wherein mixing the M samples and N assays comprises opening fluid lines to provide for fluid communication between the firsrt chambers containing one of the M samples and each of the set of second chambers containing one of the N assays.

86. The method of claim 82 wherein the first chambers are characterized by a volume of less than or equal to 100 nl.

87. The method of claim 86 wherein the volume is less than or equal to 40 nl.

88. The method of claim 82 wherein the second chambers are characterized by a volume of less than or equal to 10 nl.

89. The method of claim 88 wherein the volume is less than or equal to 2 nl.

90. The method of claim 82 wherein the closed volume is characterized by a volume of less than or equal to 100 nl.

91. The method of claim 82 further comprising thermocycling the MxN pairwise combinations.

92. The method of claim 82 wherein the M samples are provided at M sample ports of a microfluidic device.

93. The method of claim 91 wherein the MxN samples are recovered at the M sample ports of the microfluidic device.

94. The method of claim 82 wherein forming the MxN pairwise combinations is performed concurrently.

95. The method of claim 82 wherein forming the MxN pairwise combinations is performed sequentially.