CLAIMS

What is claimed is:

1. A method of adding adaptor molecules to each end of a plurality of target nucleic acids that comprise sticky ends, the method comprising:

(a) annealing adaptor molecules to the sticky ends of double-stranded target nucleic acid molecules to produce annealed adaptor-target nucleic acid molecules, wherein the adaptor molecules are:

(i) hairpin structures each comprising:

an adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a nucleotide sequence that is capable of annealing to the adaptor nucleotide sequence and is linked to

a degenerate tail sequence; or

(ii) double-stranded or single-stranded molecules each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence; and

a degenerate tail sequence on each strand, wherein double-stranded molecules each comprise two degenerate tail sequences as sticky end(s);

(b) filling any gaps in the resulting annealed adaptor-target nucleic acid molecules; and

(c) ligating any adjacent nucleotide sequences in the annealed adaptor-target nucleic acid molecules to produce adaptor-modified target nucleic acid molecules.

2. The method of claim 1, wherein the plurality of DNA molecules comprises a DNA library to be sequenced.

3. The method of any preceding claim, wherein the method comprises producing the plurality of target nucleic acid molecules that comprise sticky ends by fragmenting DNA molecules.

4. The method of claim 3, wherein the DNA molecules are fragmented by digesting them with a DNAse enzyme.

5. The method of claim 3, wherein said fragmenting of DNA molecules is followed by digesting the fragmented DNA molecules with an enzyme to produce sticky ends.

6. The method of claim 5, wherein the enzyme employed to produce sticky ends comprises a strand-specific endonuclease that does not have polymerase activity under the conditions employed in the digestion.

7. The method of any preceding claim, wherein the sticky ends of the double-stranded target nucleic acid molecules are 3' extensions.

8. The method of any preceding claim, wherein the degenerate tail sequence(s) is/are at the 3' ends of the adaptor molecules.

9. The method of any preceding claim, wherein the adaptor molecules are hairpin structures each comprising:

an adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a nucleotide sequence that is capable of annealing to the first adaptor nucleotide sequence and is linked to

a degenerate tail sequence.

10. The method of claim 9, wherein the adaptor molecules comprise:

a first type of adaptor molecule comprising a first adaptor nucleotide sequence; and

a second type of adaptor molecule comprising a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence.

11. The method of claim 9, wherein said ligating converts the annealed adaptor-target nucleic acid molecules to single-stranded circular DNA molecules.

12. The method of any of claims 1-8, wherein the adaptor molecules annealed in (a) are double-stranded molecules, each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence; and

sticky ends comprising degenerate tail sequences.

13. The method of any of claims 1-8, wherein the adaptor molecules annealed in (a) are single-stranded molecules each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence; and

a degenerate tail sequence.

14. The method of claims 10, 12, or 13, wherein the first and second adaptor sequences comprise primer binding sites that are capable of being specifically bound by DNA sequencing primers.

15. The method of claims 12 or 13, wherein said ligating converts the annealed adaptor-target nucleic acid molecules to double-stranded circular DNA molecules.

16. The method of any preceding claim, wherein the nucleotide linker comprises a nucleotide sequence selected from the group consisting of an endonuclease site, a barcode nucleotide sequence, an affinity tag, and any combination thereof.

17. The method of any preceding claim, wherein the nucleotide linker comprises a restriction enzyme site.

18. The method of any preceding claim, wherein the nucleotide linker comprises a restriction enzyme site and at least one barcode nucleotide sequence.

19. The method of claim 16, wherein the nucleotide linker comprises an endonuclease site, and the method additionally comprises digesting the single-stranded or double-stranded circular DNA molecules to produce linear DNA molecules.

20. The method of claim 16, wherein the nucleotide linker comprises a restriction enzyme site, and the method additionally comprises digesting double-stranded circular DNA molecules to produce linear DNA molecules.

21. The method of claim 19, wherein the linear DNA molecules comprise 5'-a first portion of a nucleotide linker-second adaptor nucleotide sequence-a first degenerate tail sequence-target nucleic acid molecule-a second degenerate tail sequence-first adaptor nucleotide sequence-a second portion of a nucleotide linker-3'.

22. The method of any preceding claim, wherein the method comprises:

producing the plurality of target nucleic acid molecules that comprise sticky ends by:

digesting DNA molecules with DNAse I to produce fragmented DNA molecules, and then heat inactivating the DNAse I;

digesting the fragmented DNA molecules with an nuclease having 5' to 3' exonuclease activity in the absence of deoxynucleotides to produce a plurality sticky-ended target nucleic acid molecules;

annealing the adaptors to the sticky ends of the plurality of target nucleic acid molecules, wherein the nucleotide linker of the adaptors comprises an endonuclease site;

filling any gaps and ligating any adjacent nucleotide sequences in the annealed adaptor-target nucleic acid molecules in a single reaction comprising a polymerase and a ligase to produce circular DNA molecules; and

digesting the circular DNA molecules with an endonuclease that cuts at the endonuclease site to produce linear DNA molecules.

23. The method of claim 22, wherein the nuclease having 5' to 3' exonuclease activity is Exonuclease III.

24. The method of any preceding claim, wherein the method additionally comprises sequencing the adaptor-modified target nucleic acid molecules.

25. A plurality of adaptor molecules, wherein the adaptor molecules are:

(i) hairpin structures each comprising:

an adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a nucleotide sequence that is capable of annealing to the adaptor nucleotide sequence and is linked to

a degenerate tail sequence; or

(ii) double-stranded or single-stranded molecules each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence; and

a degenerate tail sequence on each strand, wherein double-stranded molecules each comprise two degenerate tail sequences as sticky end(s).

26. The plurality of adaptor molecules of claim 25, wherein the adaptor molecules are hairpin structures each comprising:

an adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a nucleotide sequence that is capable of annealing to the first adaptor nucleotide sequence and is linked to

a degenerate tail sequence.

27. The plurality of adaptor molecules of claim 26, wherein the adaptor molecules comprise:

a first type of adaptor molecule comprising a first adaptor nucleotide sequence; and

a second type of adaptor molecule comprising a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence.

28. The plurality of adaptor molecules of claim 25, wherein the adaptor molecules annealed in (a) are double-stranded molecules, each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence; and

sticky ends comprising degenerate tail sequences.

29. The plurality of adaptor molecules of claim 25, wherein the adaptor molecules annealed in (a) are single-stranded molecules each comprising:

a first adaptor nucleotide sequence, which is linked to

a nucleotide linker, which is linked to

a second adaptor nucleotide sequence that is different from the first adaptor nucleotide sequence; and

a degenerate tail sequence.

30. The plurality of adaptor molecules of claims 27-29, wherein the first and second adaptor sequences comprise primer binding sites that are capable of being specifically bound by DNA sequencing primers.

31. The plurality of adaptor molecules of any of claims 25-30, wherein the nucleotide linker comprises a nucleotide sequence selected from the group consisting of an endonuclease site, a barcode nucleotide sequence, an affinity tag, and any combination thereof.

32. The plurality of adaptor molecules of any of claims 25-30, wherein the nucleotide linker comprises a restriction enzyme site.

33. The plurality of adaptor molecules of any of claims 25-30, wherein the nucleotide linker comprises a restriction enzyme site and at least one barcode nucleotide sequence.

34. A kit comprising the plurality of adaptor molecules of any of claims 25-33 and one or more components selected from the group consisting of a DNAse enzyme, an exonuclease, an endonuclease, a polymerase, and a ligase.

35. A method for tagging a plurality of target nucleic acids with nucleotide sequences, the method comprising:

(a) preparing a first reaction mixture for each target nucleic acid, the first reaction mixture comprising a pair of inner primers and a pair of outer primers, wherein:

(i) the inner primers comprise:

a forward, inner primer comprising a first nucleotide tag, a first barcode nucleotide sequence, and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion, a first barcode nucleotide sequence, and a second nucleotide tag; and

(ii) the outer primers comprise:

a forward, outer primer comprising a second barcode nucleotide sequence and a first nucleotide tag-specific portion; and

a reverse, outer primer comprising a second nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the inner primers; and

(b) subjecting each first reaction mixture to a reaction to produce a plurality of tagged target nucleotide sequences, each comprising 5'-second barcode nucleotide sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-3'.

36. The method of claim 35, wherein the reaction comprises amplification.

37. The method of claim 35, wherein the reaction comprises ligation.

38. The method of claim 35, wherein the outer primers additionally comprise first and second primer binding sites that are capable of being bound by DNA sequencing primers.

39. The method of claim 38, wherein the reaction produces tagged target nucleotide sequences comprising 5'-first primer binding site-second barcode nucleotide

sequence-first nucleotide tag sequence-first barcode nucleotide sequence-target nucleotide sequence-first barcode nucleotide sequence-second nucleotide tag sequence-second barcode nucleotide sequence-second primer binding site-3'.

40. A kit for performing the method of claim 35, wherein the kit comprises a polymerase and:

(i) inner primers comprising:

a forward, inner primer comprising a first nucleotide tag, a first barcode nucleotide sequence, and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion, a first barcode nucleotide sequence, and a second nucleotide tag; and

(ii) outer primers comprising:

a forward, outer primer comprising a second barcode nucleotide sequence and a first nucleotide tag-specific portion; and

a reverse, outer primer comprising a second nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the inner primers.

41. A method for tagging a plurality of target nucleic acids with nucleotide sequences, the method comprising:

(a) preparing a first reaction mixture for each target nucleic acid, the first reaction mixture comprising a pair of inner primers, a pair of stuffer primers, and a pair of outer primers, wherein:

(i) the inner primers comprise:

a forward, inner primer comprising a first nucleotide tag and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion and a second nucleotide tag;

(ii) the stuffer primers comprise:

a forward, stuffer primer comprising a third nucleotide tag, a first barcode nucleotide sequence, and a first nucleotide tag-specific portion; and

a reverse, stuffer primer comprising a second nucleotide tag-specific portion, a first barcode nucleotide sequence, a fourth nucleotide tag; and

(iii) the outer primers comprise:

a forward, outer primer comprising a second barcode nucleotide sequence and a third nucleotide tag-specific portion; and

a reverse, outer primer comprising a fourth nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the stuffer primers, which are in excess of the inner primers; and

(b) subjecting each first reaction mixture to a reaction to produce a plurality of tagged target nucleotide sequences, each comprising 5'-second barcode nucleotide sequence-third nucleotide tag sequence-first barcode nucleotide sequence-first nucleotide tag sequence-target nucleotide sequence-second nucleotide tag sequence-first barcode nucleotide sequence-fourth nucleotide tag sequence-second barcode nucleotide sequence-3'.

42. The method of claim 41, wherein the reaction comprises amplification.

43. The method of claim 41, wherein the reaction comprises ligation.

44. The method of claim 41, wherein the outer primers additionally comprise first and second primer binding sites that are capable of being bound by DNA sequencing primers.

45. The method of claim 44, wherein the reaction produces tagged target nucleotide sequences comprising 5'- first primer binding site-second barcode nucleotide sequence-third nucleotide tag sequence-first barcode nucleotide sequence-first nucleotide tag sequence-target nucleotide sequence-second nucleotide tag sequence-first barcode nucleotide sequence-fourth nucleotide tag sequence-second barcode nucleotide sequence-second primer binding site-3'.

46. A kit for performing the method of claim 41, wherein the kit comprises a polymerase and:

(i) inner primers comprising:

a forward, inner primer comprising a first nucleotide tag and a target-specific portion; and

a reverse, inner primer comprising a target-specific portion and a second nucleotide tag;

(ii) stuffer primers comprising:

a forward, stuffer primer comprising a third nucleotide tag, a first barcode nucleotide sequence, and a first nucleotide tag-specific portion; and

a reverse, stuffer primer comprising a second nucleotide tag-specific portion, a first barcode nucleotide sequence, a fourth nucleotide tag; and

(iii) outer primers comprising:

a forward, outer primer comprising a second barcode nucleotide sequence and a third nucleotide tag-specific portion; and

a reverse, outer primer comprising a fourth nucleotide tag-specific portion and a second barcode nucleotide sequence;

wherein the outer primers are in excess of the stuffer primers, which are in excess of the inner primers.

47. The kit of claims 40 or 46, wherein the outer primers additionally comprise first and second primer binding sites that are capable of being bound by DNA sequencing primers.

48. A method for combinatorial tagging of a plurality of target nucleotide sequences, the method comprising:

cutting a plurality of tagged target nucleotide sequences derived from target nucleic acids, each tagged target nucleotide sequence comprising an endonuclease site and a first barcode nucleotide sequence, wherein tagged target nucleotide sequences in the plurality comprise the same endonuclease site, but N different first barcode nucleotide sequences, wherein N is an integer greater than 1, with an endonuclease specific for the endonuclease site to produce a plurality of sticky-ended, tagged target nucleotide sequences;

ligating a plurality of adaptors comprising a second barcode nucleotide sequence and complementary sticky ends to the plurality of sticky-ended, tagged target nucleotide sequences in a first reaction mixture, wherein the plurality of adaptors comprise M different second barcode nucleotide sequences, wherein M is an integer greater than 1, to produce a plurality of combinatorially tagged target nucleotide sequences, each comprising first and second barcode nucleotide sequences, wherein the plurality comprises N × M different first and second barcode combinations.

49. The method of claim 48, wherein the endonuclease site is adjacent to the first barcode nucleotide sequence in the tagged target nucleotide sequences.

50. The method of claim 49, wherein the second barcode nucleotide sequence is adjacent to the complementary sticky end in the adaptors.

51. The method of any of claims 50-52, wherein the combinatorially tagged target nucleotide sequences comprise the first and second barcode nucleotide sequences separated by fewer than 5 nucleotides.

52. The method of any of claims 50-53, wherein the tagged target nucleotide sequences comprise first and second primer binding site having an arrangement selected from:

5'-endonuclease site-first barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site; and

5'-first primer binding site-target nucleotide sequence-second primer binding site-first barcode nucleotide sequence-endonuclease site-3'.

53. The method of claim 52, wherein the first and second primer binding sites are binding sites for DNA sequencing primers.

54. The method of claims 52 or 53, wherein the combinatorially tagged target nucleotide sequences comprise:

5'-second barcode nucleotide sequence-first barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site; or

5'-first primer binding site-target nucleotide sequence-second primer binding site-first barcode nucleotide sequence- second barcode nucleotide sequence -3'.

55. The method of any of claims 48-54, wherein the tagged target nucleotide sequences are prepared by ligating adaptors onto a plurality of target nucleic acids, wherein the adaptors comprise:

a first adaptor comprising the endonuclease site, the first barcode nucleotide sequence, the first primer binding site, and a sticky end; and

a second adaptor comprising a second primer binding site and a sticky end.

56. The method of claims 52 or 53, wherein the tagged target nucleotide sequences comprise a first additional nucleotide sequence having an arrangement selected from:

5'-endonuclease site-first barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site- first additional nucleotide sequence; and

5'- first additional nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-first barcode nucleotide sequence-endonuclease site-3'.

57. The method of claim 56, wherein the adaptors comprise a second additional nucleotide sequence and have the arrangement: 5'-second additional nucleotide sequence-second barcode nucleotide sequence-complementary sticky end-3'.

58. The method of claim 57, wherein the combinatorially tagged target nucleotide sequences comprise:

5'-second additional nucleotide sequence-second barcode nucleotide sequence-first barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-first additional nucleotide sequence; and

5'- second additional nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-first barcode nucleotide sequence-second barcode nucleotide sequence-first additional nucleotide sequence -3'.

59. The method of claims 56-58, wherein the first and/or second additional nucleotide sequences comprise a primer binding site.

60. The method of any of claims 56-59, wherein the tagged target nucleotide sequences are prepared by ligating adaptors onto a plurality of target nucleic acids, wherein the adaptors comprise:

a first adaptor comprising the endonuclease site, the first barcode nucleotide sequence, the first primer binding site, and a sticky end; and

a second adaptor comprising a first additional nucleotide sequence, a second primer binding site, and a sticky end.

61. A plurality of adaptors comprising:

a plurality of first adaptors, each comprising the same endonuclease site, N different barcode nucleotide sequences, wherein M is an integer greater than 1, a first primer binding site and a sticky end;

a second adaptor comprising a second primer binding site and a sticky end; and

a plurality of third adaptors comprising a second barcode nucleotide sequence and sticky ends complementary to those produced upon cutting the first adaptors at the endonuclease site, wherein the plurality of third adaptors comprise M different second barcode nucleotide sequences, wherein M is an integer greater than 1.

62. A kit comprising the adaptors of claim 61 and one or more components selected from the group consisting of: an endonuclease specific for the endonuclease site in the first adaptors and a ligase.

63. A method for combinatorial tagging of a plurality of target nucleotide sequences, the method comprising:

annealing a plurality of barcode primers to a plurality of tagged target nucleotide sequences derived from target nucleic acids, wherein:

each tagged target nucleotide sequence comprises a nucleotide tag at one end and a first barcode nucleotide sequence, wherein tagged target nucleotide sequences in the plurality comprise the same nucleotide tag, but N different first barcode nucleotide sequences, wherein N is an integer greater than one; and

each barcode primer comprises:

a first tag-specific portion linked to;

a second barcode nucleotide sequence linked to;

a second tag-specific portion;

wherein the barcode primers in the plurality each comprise the same first and second tag-specific portions, but M different second barcode nucleotide sequences, wherein M is an integer greater than one; and

amplifying the tagged target nucleotide sequences in a first reaction mixture to produce a plurality of combinatorially tagged target nucleotide sequences, each comprising first and second barcode nucleotide sequences, wherein the plurality comprises N × M different first and second barcode combinations.

64. The method of claim 63, wherein the first barcode nucleotide sequence is separated from the nucleotide tag by the target nucleotide sequence.

65. The method of claims 63 or 64, wherein the first tag-specific portion of the barcode primer anneals to a 5' portion of the nucleotide tag, and the second tag-specific portion of the barcode primer anneals to an adjacent 3' portion of the nucleotide tag, and the second barcode nucleotide sequence does not anneal to the nucleotide tag, forming a loop between the annealed first and second tag-specific portions.

66. The method of any of claims 63-65, wherein the tagged nucleotide sequences additionally comprise a primer binding site between the target nucleotide sequence and the first barcode nucleotide sequence.

67. The method of any of claims 63-66, wherein the first and second tag-specific portions of the barcode primer are sufficiently long to serve as primer binding sites.

68. The method of claims 66 or 67, wherein binding sites are binding sites for DNA sequencing primers.

69. The method of any of claims 66-68, wherein the combinatorially tagged target nucleotide sequences comprise 5'-first tag-specific portion-second barcode nucleotide sequence-second tag-specific portion-target nucleotide sequence-primer binding site-first barcode nucleotide sequence-3'.

70. The method of claims 66-69, wherein the tagged target nucleotide sequences comprise a first additional nucleotide sequence having the arrangement: 5'-nucleotide tag-target nucleotide sequence-primer binding site-first barcode nucleotide sequence-first additional nucleotide sequence-3'.

71. The method of claim 70, wherein the barcode primers additionally comprise a second additional nucleotide sequence and have the arrangement: 5'-second additional nucleotide sequence-first tag-specific portion-second barcode nucleotide sequence-second tag-specific portion-3'.

72. The method of claim 71, wherein the combinatorially tagged target nucleotide sequences comprise 5'- second additional nucleotide sequence-first tag-specific portion-second barcode nucleotide sequence-second tag-specific portion-target nucleotide sequence-primer binding site-first barcode nucleotide sequence-first additional nucleotide sequence-3'.

73. The method of claims 70-72, wherein the first and/or second additional nucleotide sequences comprise a primer binding site.

74. The method of any of claims 66-73, wherein the nucleotide tag comprises a transposon end, which is incorporated into the tagged target nucleotide sequences using a transposase.

75. The method of any of claims 35-74, wherein the reaction mixtures are prepared in separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns, wherein the combination of the first and second barcode nucleotide sequences in each tagged target nucleotide sequence identifies the row and column of the compartment that was the source of the tagged target nucleotide sequence.

76. The method of claim 75, wherein the method comprises recovering the tagged target nucleotide sequences from the microfluidic device.

77. The method of of claim 76, wherein said recovering comprises pooling tagged target nucleotide sequences from compartments in a row or in a column to produce an assay pool.

78. The method of claim 77, wherein the method comprises further analyzing the assay pool by nucleic acid amplification.

79. The method of claim 77, wherein the method comprises further analyzing the assay pool by nucleic acid sequencing.

80. The method of claims 78 or 79, wherein said further analysis comprises determining whether a tagged target nucleotide sequence comprising a selected combination of first and second barcode nucleotide sequences is present in the assay pool, or aliquot thereof.

81. A kit for performing the method of claim 63, the kit comprising:

one or more nucleotide tag(s); and

a plurality of barcode primers, wherein each barcode primer comprises:

a first portion that is specific for a first portion of the nucleotide tag(s) linked to;

a barcode nucleotide sequence that does not anneal to the nucleotide tag(s) linked to;

a second portion that is specific for a second portion of the nucleotide tag(s);

wherein the barcode primers in the plurality each comprise the same first and second tag-specific portions, but M different second barcode nucleotide sequences, wherein M is an integer greater than one.

82. The kit of claim 81, wherein the nucleotide tag(s) comprise transposon ends, and the kit additionally comprises a transposase.

83. The kit of claims 81 or 82, wherein the kit additionally comprises a polymerase.

84. An assay method for detecting a plurality of target nucleic acids, the method comprising:

preparing M first reaction mixtures that will be pooled prior to assay, wherein M is an integer greater than 1, and each first reaction mixture comprises:

sample nucleic acid(s);

a first, forward primer comprising a target-specific portion;

a first, reverse primer comprising a target-specific portion, wherein the first, forward primer or the first, reverse primer additionally comprises a barcode nucleotide sequence, wherein each barcode nucleotide sequence in each of said M reaction mixtures is different;

subjecting each first reaction mixture to a first reaction to produce a plurality of barcoded target nucleotide sequences, each comprising a target nucleotide sequence linked to a barcode nucleotide sequence.

for each of the M first reaction mixtures, pooling the barcoded target nucleotide sequences to form an assay pool;

subjecting the assay pool, or one or more aliquots thereof, to a second reaction using unique pairs of second primers, wherein each second primer pair comprises:

a second, forward or a reverse primer that anneals to a target nucleotide sequence; and

a second, reverse or a forward primer, respectively, that anneals to a barcode nucleotide sequence; and

for each unique, second primer pair, determining whether a reaction product is present in the assay pool, or aliquot thereof, whereby the presence of a reaction product indicates the presence of a particular target nucleic acid in a particular first reaction mixture.

85. An assay method for detecting a plurality of target nucleic acids, the method comprising:

preparing M first reaction mixtures that will be pooled prior to assay, wherein M is an integer greater than 1, and each first reaction mixture comprises:

sample nucleic acid(s)

a first, forward primer comprising a target-specific portion;

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

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

subjecting each first reaction mixture to a first reaction to produce a plurality of barcoded target nucleotide sequences, each comprising a target nucleotide sequence linked to a nucleotide tag, which is linked to a barcode nucleotide sequence.

for each of the M reaction mixtures, pooling the barcoded target nucleotide sequences to form an assay pool;

subjecting the assay pool, or one or more aliquots thereof, to a second reaction using unique pairs of second primers, wherein each second primer pair comprises:

a second, forward or a reverse primer that anneals to a target nucleotide sequence; and

a second, reverse or a forward primer, respectively, that anneals to a barcode nucleotide sequence; and

for each unique, second primer pair, determining whether a reaction product is present in the assay pool, or aliquot thereof, whereby the presence of a reaction product indicates the presence of a particular target nucleic acid in a particular first reaction mixture.

86. The method of any of claims 84 or 85, wherein the method additionally comprises preparing M × N first reaction mixtures, wherein N is an integer greater than 1, and:

each first reaction mixture comprises a pair of first, forward and reverse primers that is specific for a different target nucleic acid:

said pooling comprises preparing N assay pools of M first reaction mixtures, wherein each barcoded target nucleotide sequence in an assay pool comprises a different barcode nucleotide sequence; and

the second reaction is carried out in each of the N assay pools, with each assay pool being separate from every other assay pool.

87. The method of any of claims 84-86, wherein the first reaction comprises amplification.

88. The method of any of claims 84-87, wherein the second reaction comprises amplification.

89. The method of any of claims 35-86, wherein each first reaction mixture comprises target nucleic acids from a single particle.

90. The method of claim 89, wherein the single particle comprises a particle selected from the group consisting of a cell, an organelle, a vesicle, a chromosome, a chromosomal fragment, and a particle produced using a chemical crosslinker.

91. The method of claim 89, wherein the single particle comprises a single cell.

92. The method of any of claims 84-91, wherein the first reaction mixtures are prepared in separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns.

93. The method of claim 86, wherein:

the first reaction mixtures are prepared in separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns;

each of the N assay pools is obtained by pooling the first reaction mixtures in a row or a column of the device; and

the barcode nucleotide sequence in each barcoded target nucleotide sequence, taken with the identity of the assay pool, identifies the row and column of the compartment that was the source of the barcoded target nucleotide sequence.

94. The method of any of claims 84-93, wherein the second reaction mixtures are prepared in separate compartments of a microfluidic device, the separate compartments being arranged as an array defined by rows and columns.

95. The method of claim 94, wherein the first reaction mixtures are prepared in separate compartments of a first microfluidic device, the second reaction

mixtures are prepared in separate compartments of a second microfluidic device, wherein the second microfluidic device is different from the first.

96. The method of claims 92 or 93, wherein particles to be analyzed are distributed into the compartments of the device.

97. The method of claim 96, wherein the number of particles in each compartment is determined by brightfield microscopy or fluorescence microscopy.

98. The method of claim 96, wherein a stain, dye, or label is employed to detect the number of particles in each compartment.

99. The method of claim 98, wherein the particles are cells, and a cell membrane-permeant nucleic acid dye is employed to detect the number of cells in each compartment.

100. The method of claim 98, wherein the particles are cells, and a labeled antibody specific for a cell-surface marker is employed to detect the number of cells in each compartment.

101. The method of claim 96, wherein the method comprises:

determining whether any compartment comprises more than a single particle; and

not further analyzing, or disregarding, results from, any compartment that comprises more than a single particle.

102. The method of claim 96, wherein the method comprises:

determining whether any compartment comprises a particle having a particular characteristic; and

further analyzing, or only considering results from, any compartment that comprises a particle having the particular characteristic.

103. The method of claim 102, wherein the characteristic is selected from the group consisting of a specific genomic rearrangement, copy number variation, or polymorphism; expression of a specific gene; and expression of a specific protein.

104. The method of any of claims 89-103, wherein at least one reaction is performed with intact particles.

105. The method of any of claims 89-103, wherein at least one reaction is performed with disrupted particles.

106. The method of any of claims 89-105, wherein the particles are treated with an agent that elicits biological response prior to performing the plurality of first reactions.

107. The method of any of claims 84-106, wherein said determining whether a reaction product is present in the assay pool, or aliquot thereof, is carried out by polymerase chain reaction (PCR).

108. The method of any of claims 84-106, wherein said determining whether a reaction product is present in the assay pool, or aliquot thereof, is carried out by ligase chain reaction (LCR).

109. The method of claims 84-108, wherein said determining whether a reaction product is present in the assay pool, or aliquot thereof, is carried out by real-time detection.

110. The method of claim 109, wherein said determining whether a reaction product is present in the assay pool, or aliquot thereof, is carried out by an assay employing a labeled probe and an unlabeled probe, wherein the simultaneous hybridization of the probes to a reaction product results in the formation of a flap at the 5'end of the labeled probe, and cleavage of the flap produces a signal.

111. The method of claim 108, wherein cleavage of the flap separates a fluorophore from a quencher.

112. The method of claim 108, wherein a portion of the labeled probe is specific for a barcode nucleotide sequence in the reaction product.

113. The method of claim 108, wherein said determining whether a reaction product is present in the assay pool, or aliquot thereof, is carried out by melting temperature analysis comprising detection at a temperature at which:

reaction products are substantially double-stranded and capable of producing signal in the presence of a double-stranded DNA-binding dye; and

primers are substantially single-stranded and incapable of producing signal in the presence of a double-stranded DNA-binding dye.

114. The method of any of claims 84-113, wherein the method comprises sequencing the reaction products.

115. The method of any of claims 35-114, wherein the method comprises determining the amount of at least one target nucleic acid in the first reaction mixtures.

116. The method of any of claims 35-115, wherein the method comprises determining the copy number(s) of one or more DNA molecule(s) in the first reaction mixtures.

117. The method of claim 116, wherein tagged or barcoded target nucleotide sequences are produced by fewer than 20 cycles of PCR.

118. The method of any of claims 35-117, wherein the method comprises determining the genotype(s) at one or more loci in the first reaction mixtures.

119. The method of any of claims 35-118, wherein the method comprises determining a haplotype for a plurality of loci in the first reaction mixtures.

120. The method of claim 119, wherein the method comprises:

condensing chromosomes; and

distributing chromosomes into first reaction mixtures to produce a plurality of first reaction mixtures that comprise a single chromosome;

wherein the reaction comprises sequencing a plurality of loci in each of the first reaction mixtures.

121. The method of any of claims 35-120, wherein the method comprises determining the expression level(s) of one or more RNA molecule(s) in the first reaction mixtures.

122. The method of claim 121, wherein tagged or barcoded target nucleotide sequences are produced by fewer than 20 cycles of RT-PCR.

123. The method of any of claims 35-122, wherein the method comprises determining the nucleotide sequence(s) of one or more RNA molecule(s) in the first reaction mixtures.

124. The method of any of claims 35-123, wherein the method comprises:

performing a plurality of reactions in each first reaction mixture, wherein one of the plurality of reactions comprises amplification to produce a tagged or barcoded target nucleotide sequence;

analyzing the results of the plurality of reactions; and

associating the results of the analysis with each first reaction mixture.

125. The method of claim 124, wherein the results of the analysis indicate the presence of two or more of a variation selected from the group consisting of:

a copy number variation;

a mutation;

an expression level variation;

a splice variant;

wherein the presence of said two or more variations is correlated with a phenotype.

126. The method of claim 125, wherein the phenotype is selected from the risk, presence, severity, prognosis, and responsiveness to a specific therapy of a disease.

127. The method of claim 126, wherein the phenotype comprises resistance to a drug.

128. The method of claim 124, where the results of analysis indicate the occurrence of genomic recombination.

129. The method of claim 124, where the results of analysis indicate co-expression of particular splice variants.

130. The method of claim 124, where the results of analysis indicate co-expression of particular light and heavy chains in B cells.

131. The method of claim 124, where the results of analysis indicate the presence of a particular pathogen in a particular host cell.

132. A method for amplifying a target nucleic acid, the method comprising:

amplifying a target nucleic acid using:

a set of inner primers, wherein the set comprises:

an inner, forward primer comprising a target-specific portion and a first primer binding site;

an inner, reverse primer comprising a target-specific portion and a second primer binding site, wherein the first and second primer binding sites are different;

a first set of outer primers, wherein the set comprises:

a first outer, forward primer comprising a portion specific for the first primer binding site; and

a first outer, reverse primer comprising a barcode nucleotide sequence and a portion specific for the second primer binding site;

a second set of outer primers, wherein the set comprises:

a second outer, forward primer comprising a barcode nucleotide sequence and a portion specific for the first primer binding site; and

a second outer, reverse primer comprising a portion specific for the second primer binding site;

to produce two target amplicons, wherein:

a first target amplicon comprises 5'-first primer binding site-target nucleotide sequence-second primer binding site-barcode nucleotide sequence-3'; and

a second target amplicon comprises 5'-barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-3'.

133. The method of claim 132, wherein the barcode nucleotide sequence in each target amplicon is the same, and wherein each target amplicon comprises only one barcode nucleotide sequence.

134. The method of claim 132, wherein the first and second primer binding sites are binding sites for DNA sequencing primers.

135. The method of any of claims 132-134, wherein the outer primers each additionally comprise an additional nucleotide sequence, wherein:

the first outer, forward primer comprises a first additional nucleotide sequence, and the first outer, reverse primer comprises a second additional nucleotide sequence; and

the second outer, forward primer comprises the second additional nucleotide sequence, and the second outer, reverse primer comprises the first additional nucleotide sequence; and

the first and second additional nucleotide sequences are different; and

said amplifying produces two target amplicons, wherein:

a first target amplicon comprises 5'-first additional nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-barcode nucleotide sequence-second additional nucleotide sequence-3'; and

a second target amplicon comprises 5'-second additional nucleotide sequence-barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-first additional nucleotide sequence 3'.

136. The method of claim 135, wherein the first or second additional nucleotide sequence comprises a primer binding site.

137. The method of claim 136, wherein the first and second additional nucleotide sequences comprise a primer binding site.

138. The method of claims 135-137, wherein the first set of outer primers comprises PE1-CS1 and PE2-BC-CS2, and the second set of outer primers comprises PE1-CS2 and PE2-BC-CS1 (Table, Example 9).

139. The method of claim 132, wherein said amplifying is carried out in a single amplification reaction.

140. The method of claim 132, wherein said amplifying comprises employing the inner primers in a first amplification reaction and employing the outer primers in a second amplification reaction, wherein said second amplification reaction is separate from the first.

141. The method of claim 140, wherein the second amplification reaction comprises two separate amplification reactions, wherein one employs the first set of outer primers and the other employs the second set of outer primers.

142. The method of claim 141, wherein the target amplicons produced in the two separate second amplification reactions are pooled.

143. The method of any of claims 132-142, wherein the method comprises amplifying a plurality of target nucleic acids.

144. The method of claim 143, wherein the plurality of target nucleic acids is selected from the group consisting of genomic DNA, cDNA, fragmented DNA, DNA reverse-transcribed from RNA, a DNA library, and nucleic acids is extracted or amplified from a cell, a bodily fluid or a tissue sample.

145. The method of claim 144, wherein the plurality of target nucleic acids is amplified from a formalin-fixed, paraffin-embedded tissue sample.

146. The method of any of claims 132-145, wherein the method additionally comprises sequencing the target amplicons.

147. The method of claim 143, wherein the method comprises amplifying the target amplicons using primers that bind to the first and second additional nucleotide sequences to produce templates for DNA sequencing.

148. The method of claim 147, wherein one or both primers that bind to the first and second additional nucleotide sequences are immobilized on a substrate.

149. The method of claims 143 or 147, wherein the amplification is carried out by isothermal nucleic acid amplification.

150. The method of claim 147, wherein the method comprises performing DNA sequencing using the templates and primers that bind to the first and second primer binding sites and prime sequencing of the target nucleotide sequence(s).

151. The method of claim 150, wherein the primers that bind to the first and second primer binding sites and prime sequencing of the target nucleotide sequence(s) are present in substantially equal amounts.

152. The method of claim 147, wherein the method comprises performing DNA sequencing using the templates and primers that bind to the first and second primer binding sites and prime sequencing of the barcode nucleotide sequences(s).

153. The method of claim 152, wherein the primers that bind to the first and second primer binding sites and prime sequencing of the barcode nucleotide sequences(s) are present in substantially equal amounts.

154. The method of claim 150, wherein the method comprises performing DNA sequencing using the templates and primers that bind to the first and second primer binding sites and prime sequencing of the barcode nucleotide sequences(s), wherein said primers are reverse complements of the primers that prime sequencing of the target nucleotide sequences.

155. The method of claim 154, wherein the primers employed to prime sequencing of the target nucleotide sequence(s) and barcode nucleotide sequence(s) comprise CS1, CS2, CS1rc, and CS2rc (Table 4, Example 9).

156. The method of any of claims 132-155, wherein the barcode nucleotide sequence is selected so as to avoid substantial annealing to the target nucleic acids.

157. The method any of claims 132-156, wherein the barcode nucleotide sequence identifies a particular sample.

158. The method of claim 147, wherein at least 50 percent of the sequences determined from DNA sequencing are present at greater than 50 percent of the average number of copies of sequences and less than 2-fold the average number of copies of sequences.

159. The method of claim 147, wherein at least 70 percent of the sequences determined from DNA sequencing are present at greater than 50 percent of the average number of copies of sequences and less than 2-fold the average number of copies of sequences.

160. The method of claim 147, wherein at least 90 percent of the sequences determined from DNA sequencing are present at greater than 50 percent of the average number of copies of sequences and less than 2-fold the average number of copies of sequences.

161. The method of any of claims 132-160, wherein the average length of the target amplicons is less than 200 bases.

162. The method of any of claims 132-161, wherein the first amplification reaction is carried out in a volume in the range of about 1 picoliter to about 50 nanoliters.

163. The method of any of claims 132-162, wherein the first amplification reaction is carried out in a volume in the range of about 5 picoliters to about 25 nanoliters.

164. The method of any of claims 132-163, wherein the first amplification reaction(s) is/are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification.

165. The method of claim 164, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

166. The method of any of claims 132-163, wherein the first amplification reaction(s) is/are carried out in (a) fluid droplet(s).

167. The method of claim 166, wherein a plurality of first amplification reactions are carried out in fluid droplets in an emulsion.

168. A kit for use in amplifying a target nucleic acid, the kit comprising:

a first set of outer primers, wherein the set comprises:

a first outer, forward primer comprising a portion specific for a first primer binding site; and

a first outer, reverse primer comprising a barcode nucleotide sequence and a portion specific for a second primer binding site, wherein the first and second primer binding sites are different;

a second set of outer primers, wherein the set comprises:

a second outer, forward primer comprising a barcode nucleotide sequence and a portion specific for the first primer binding site; and

a second outer, reverse primer comprising a portion specific for the second primer binding site.

169. The kit of claim 168, wherein the first and second primer binding sites are binding sites for DNA sequencing primers.

170. The kit of claim 168 or 169, wherein the outer primers each additionally comprise an additional nucleotide sequence, wherein:

the first outer, forward primer comprises a first additional nucleotide sequence, and the first outer, reverse primer comprises a second additional nucleotide sequence; and

the second outer, forward primer comprises the second additional nucleotide sequence, and the second outer, reverse primer comprises the first additional nucleotide sequence; and the first and second additional nucleotide sequences are different.

171. The kit of claim 170, wherein the first set of outer primers comprises PE1-CS1 and PE2-BC-CS2, and the second set of outer primers comprises PE1-CS2 and PE2-BC-CS1 (Table, Example 9).

172. The kit of claim 168, additionally comprising:

a set of inner primers, wherein the set comprises:

an inner, forward primer comprising a target-specific portion and the first primer binding site; and

an inner, reverse primer comprising a target-specific portion and the second primer binding site.

173. The kit of claim 172, comprising a plurality of sets of inner primers, each specific for a different target nucleic acid.

174. The kit of any of claims 168-172, additionally comprising DNA sequencing primers that bind to the first and second primer binding sites and prime sequencing of the target nucleotide sequence(s).

175. The kit of any of claims 168-174, additionally comprising DNA sequencing primers that bind to the first and second primer binding sites and prime sequencing of the barcode nucleotide sequence(s).

176. The kit of claim 175, wherein the primers that bind to the first and second primer binding sites and prime sequencing of the barcode nucleotide sequences(s) are reverse complements of the primers that prime sequencing of the target nucleotide sequences.

177. The kit of claim 176, wherein the primers employed to prime sequencing of the target nucleotide sequence(s) and barcode nucleotide sequence(s) comprise CS1, CS2, CS1rc, and CS2rc (Table 4, Example 9).

178. A method for detecting, and/or quantifying the relative amounts of, at least two different target nucleic acids in a nucleic acid sample, the method comprising:

producing first and second tagged target nucleotide sequences from first and second target nucleic acids in the sample,

the first tagged target nucleotide sequence comprising a first nucleotide tag; and

the second tagged target nucleotide sequence comprising a second nucleotide tag, wherein the first and second nucleotide tags are different;

subjecting the tagged target nucleotide sequences to:

a first primer extension reaction using a first primer that anneals to the first nucleotide tag; and

a second primer extension reaction using a second primer that anneals to the second nucleotide tag; and

detecting and/or quantifying:

a signal that indicates extension of the first primer; and

a signal that indicates extension of the second primer

wherein the a signal for a given primer indicates the presence, and/or relative amount of, the corresponding target nucleic acid.

179. The method of claim 178, wherein the first and second tagged target nucleotide sequences comprise adaptors for DNA sequencing at the ends of each molecule.

180. The method of claim 178 or 179, wherein the first and second tagged target nucleotide sequences are produced by amplifying first and second target nucleic acids with first and second primer pairs, respectively, wherein at least one primer in the first primer pair comprises a first nucleotide tag and at least one primer in the second primer pair comprises a second nucleotide tag.

181. The method of claim 180, wherein one primer in each primer pair comprises 5'-(DNA sequencing adaptor)-(nucleotide tag)-(target-specific portion)-3' and the other primer in each primer pair comprises 5'-(DNA sequencing adaptor)-(target-specific portion)-3'.

182. The method of claim 180, wherein the tagged target nucleotide sequences are further amplified prior to primer extension.

183. The method of claim 182, wherein said further amplification comprises emulsion amplification or bridge amplification.

184. The method of any of claims 178-183, wherein the first and second primer extension reactions are carried out sequentially in at least two cycles of primer extension, wherein:

a first cycle of primer extension is carried out using the first primer that anneals to the first nucleotide tag;

a second cycle of primer extension is carried out using the second primer that anneals to the second nucleotide tag;

all deoxynucleoside triphosphates are provided in each cycle of primer extension;

the incorporation of any deoxynucleoside triphosphate into a DNA molecule produces a detectable signal; and

the signal detected in the first cycle indicates the presence, and/or relative amount, of the first target nucleic acid in the nucleic acid sample, and

the signal detected in the second cycle indicates the presence, and/or relative amount, of the second target nucleic acid in the nucleic acid sample.

185. The method of any of claims 178-184, wherein the detectable signal comprises pyrophosphate release.

186. The method of claims 184 or 185, wherein the tagged target nucleotide sequences are further amplified by emulsion PCR prior to primer extension.

187. The method of claims 178 or 182, wherein the first and second primer extension reactions are carried out by oligonucleotide ligation and detection, and wherein:

the ligation of a labeled di-base oligonucleotide to the first and/or second primer(s) produces a detectable signal; and

the total signal detected for a particular primer indicates the presence, and/or relative amount, of the corresponding target nucleic acid in the nucleic acid sample.

188. The method of claim 187, wherein the ligation of a labeled di-base oligonucleotide to the first primer produces the same detectable signal as the ligation of a labeled di-base oligonucleotide to the second primer, and the first and second primer extension reactions are carried out separately.

189. The method of claim 188, wherein the first and second primer extension reactions are carried out in sequential cycles.

190. The method of claim 187, wherein the ligation of a labeled di-base oligonucleotide to the first primer produces a different detectable signal than the ligation of a labeled di-base oligonucleotide to the second primer.

191. The method of claim 190, wherein the first and second primer extension reactions are carried out simultaneously, in one reaction mixture.

192. The method of any of claims 187-191, wherein the detectable signal is a fluorescent signal.

193. The method of any of claims 187-192, wherein the tagged target nucleotide sequences are further amplified by emulsion PCR prior to primer extension.

194. The method of claims 178 or 182, wherein the first and second primer extension reactions comprise sequencing-by-synthesis, wherein:

each deoxynucleoside triphosphates is labeled with a distinct, base-specific label;

the incorporation of a deoxynucleoside triphosphate into a DNA molecule produces a base-specific detectable signal; and

the total signal detected for a particular primer indicates the presence, and/or relative amount, of the corresponding target nucleic acid in the nucleic acid sample.

195. The method of claim 194, wherein the extension of the first primer produces the same detectable signal as the extension of the second primer, and the first and second primer extension reactions are carried out separately.

196. The method of claim 195, wherein the first and second primer extension reactions are carried out in sequential cycles.

197. The method of claim 194, wherein the extension of the first primer produces a different detectable signal than the extension of the second primer.

198. The method of claim 197, wherein the first and second primer extension reactions are carried out simultaneously, in one reaction mixture.

199. The method of any of claims 194-197, wherein the detectable signal is a fluorescent signal.

200. The method of any of claims 194-199, wherein the tagged target nucleotide sequences are further amplified by bridge PCR prior to primer extension.

201. The method of claim 182, wherein amplification produces clonal populations of tagged target nucleotide sequences that are, or become, located at discrete reaction sites.

202. The method of claim 201, wherein the number of reaction sites comprising the first nucleotide tag relative to the number of reaction sites comprising the second nucleotide tag indicates the amount of the first target nucleic acid relative to the second target nucleic acid in the sample.

203. The method of claims 201 or 202, wherein said detecting and/or quantifying comprises:

detecting and comparing the total signal from all reaction sites comprising the first nucleotide tag with the total signal from all reaction sites comprising the second nucleotide tag; or

detecting and comparing the number of reaction sites comprising the first nucleotide tag with the number of reaction sites comprising the second nucleotide tag.

204. The method of claim 203, wherein said comparing comprises determining a ratio.

205. The method of claims 179-187, 189-194, and 196-204, wherein the first nucleotide tag comprises a poly-(first nucleotide) sequence, and the second nucleotide tag comprises a poly-(second nucleotide) sequence, wherein the first and second nucleotides are different.

206. The method of any of claims 178-205, wherein the first and second target nucleic acids are selected from:

two different alleles of a polymorphic site;

a target nucleic acid that may be present in the nucleic acid sample at an altered copy number and a reference target nucleic acid that is expected to be present in the sample at a normal copy number;

target nucleic acids on a single chromosome; and

target nucleic acids on different chromosomes.

207. The method of claim 206, wherein the first and second target nucleic acids comprise a mutant allele and a wild type allele.

208. The method of any of claims 178-207, the method comprising:

producing three or more tagged target nucleotide sequences from three or more target nucleic acids in the sample

the tagged target nucleotide sequences are subjected to three or more primer extension reactions, each using a primer that anneals to a distinct nucleotide tag; and

a signal is detected and/or quantified for the extension of each primer.

209. The kit of any of claims 34, 40, 47, 62, 81-83, and 168-177, wherein the kit additionally comprises a matrix-type microfluidic device.

CLAIMS

What is claimed is:

1. A method for amplifying a target nucleic acid, the method comprising:

amplifying a target nucleic acid using:

a set of inner primers, wherein the set comprises:

an inner, forward primer comprising a target-specific portion and a first primer binding site;

an inner, reverse primer comprising a target-specific portion and a second primer binding site, wherein the first and second primer binding sites are different;

a first set of outer primers, wherein the set comprises:

a first outer, forward primer comprising a portion specific for the first primer binding site; and

a first outer, reverse primer comprising a barcode nucleotide sequence and a portion specific for the second primer binding site;

a second set of outer primers, wherein the set comprises:

a second outer, forward primer comprising a barcode nucleotide sequence and a portion specific for the first primer binding site; and

a second outer, reverse primer comprising a portion specific for the second primer binding site;

to produce two target amplicons, wherein:

a first target amplicon comprises 5′-first primer binding site-target nucleotide sequence-second primer binding site-barcode nucleotide sequence-3′; and

a second target amplicon comprises 5′-barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-3′;

wherein the method comprises amplifying a plurality of target nucleic acids and produces target amplicons having common first and common second primer binding sites, wherein at least some target amplicons have different barcode nucleotide sequences.

2. The method of claim 1, wherein the barcode nucleotide sequence in each target amplicon produced from a given target nucleic acid is the same, and wherein each target amplicon comprises only one barcode nucleotide sequence.

3. The method of claim 1, wherein the first and second primer binding sites are binding sites for DNA sequencing primers.

4. The method of claim 1, wherein the outer primers each additionally comprise an additional nucleotide sequence, wherein:

the first outer, forward primer comprises a first additional nucleotide sequence, and the first outer, reverse primer comprises a second additional nucleotide sequence; and

the second outer, forward primer comprises the second additional nucleotide sequence, and the second outer, reverse primer comprises the first additional nucleotide sequence; and

the first and second additional nucleotide sequences are different; and

said amplifying produces two target amplicons, wherein:

a first target amplicon comprises 5′-first additional nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-barcode nucleotide sequence-second additional nucleotide sequence-3′; and

a second target amplicon comprises 5′-second additional nucleotide sequence-barcode nucleotide sequence-first primer binding site-target nucleotide sequence-second primer binding site-first additional nucleotide sequence 3′.

5. The method of claim 4, wherein the first or second additional nucleotide sequence comprises a primer binding site.

6. The method of claim 5, wherein the first and second additional nucleotide sequences each comprise a primer binding site.

7. The method of claim 4, wherein the first set of outer primers comprises PE1-CS1 (SEQ ID NO:9) and PE2-BC-CS2 (SEQ ID NO:10), and the second set of outer primers comprises PE1-CS2 (SEQ ID NO:11) and PE2-BC-CS1 (SEQ ID NO:12).

8. The method of claim 1, wherein said amplifying is carried out in a single amplification reaction.

9. The method of claim 1, wherein said amplifying comprises employing the inner primers in a first amplification reaction and employing the outer primers in a second amplification reaction, wherein said second amplification reaction is separate from the first.

10. The method of claim 9, wherein the second amplification reaction comprises two separate amplification reactions, wherein one employs the first set of outer primers and the other employs the second set of outer primers.

11. The method of claim 10, wherein the target amplicons produced in the two separate second amplification reactions are pooled.

12. The method of claim 1, wherein the plurality of target nucleic acids is selected from the group consisting of genomic DNA, cDNA, fragmented DNA, DNA reverse-transcribed from RNA, a DNA library, and nucleic acids extracted or amplified from a cell, a bodily fluid or a tissue sample.

13. The method of claim 12, wherein the plurality of target nucleic acids is amplified from a formalin-fixed, paraffin-embedded tissue sample.

14. The method of claim 1, wherein the method additionally comprises sequencing the target amplicons.

15. The method of claim 1, wherein the method comprises amplifying the target amplicons using primers that bind to the first and second additional nucleotide sequences to produce templates for DNA sequencing.

16. The method of claim 15, wherein one or both primers that bind to the first and second additional nucleotide sequences are immobilized on a substrate.

17. The method of claim 1, wherein the amplification is carried out by isothermal nucleic acid amplification.

18. The method of claim 15, wherein the method comprises performing DNA sequencing using the templates and target sequencing primers that bind to the first and second primer binding sites, wherein said target sequencing primers prime sequencing of the target nucleotide sequence(s).

19. The method of claim 18, wherein the target sequencing primers are present in substantially equal amounts so as to produce both 5′ and 3′ DNA sequence information from each target nucleotide sequence.

20. The method of claim 15, wherein the method comprises performing DNA sequencing using the templates and barcode sequencing primers that bind to the first and second primer binding sites, wherein said barcode sequencing primers prime sequencing of the barcode nucleotide sequences(s).

21. The method of claim 20, wherein the barcode sequencing primers are present in substantially equal amounts so as to produce barcode sequences from each of the first and second target amplicons.

22. The method of claim 18, wherein the method comprises performing DNA sequencing using the templates and barcode sequencing primers that are reverse complements of the target sequencing primers.

23. The method of claim 22, wherein the target sequencing primers and the barcode sequencing primers comprise CS1 (SEQ ID NO:13), CS2 (SEQ ID NO:14), CS1rc (SEQ ID NO:15), and CS2rc (SEQ ID NO:16).

24. The method of claim 1, wherein the barcode nucleotide sequence is selected so as to avoid substantial annealing to the target nucleic acids.

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

26. The method of claim 15, wherein for at least 50 percent of the sequences determined from DNA sequencing, each determined sequence is present at greater than 50 percent of the average number of copies of that sequence and less than 2-fold said average number of copies.

27. The method of claim 15, wherein for at least 70 percent of the sequences determined from DNA sequencing, each determined sequence is present at greater than 50 percent of the average number of copies of that sequence and less than 2-fold said average number of copies.

28. The method of claim 15, wherein for at least 90 percent of the sequences determined from DNA sequencing, each determined sequence is present at greater than 50 percent of the average number of copies of that sequence and less than 2-fold said average number of copies of sequences.

29. The method of claim 1, wherein the average length of the target amplicons is less than 200 bases.

30. The method of claim 1, wherein the first amplification reaction is carried out in a volume in the range of about 1 picoliter to about 50 nanoliters.

31. The method of claim 1, wherein the first amplification reaction is carried out in a volume in the range of about 5 picoliters to about 25 nanoliters.

32. The method of claim 1, wherein the first amplification reaction(s) is/are formed in, or distributed into, separate compartments of a microfluidic device prior to amplification.

33. The method of claim 32, wherein the microfluidic device is fabricated, at least in part, from an elastomeric material.

34. The method of claim 1, wherein the first amplification reaction(s) is/are carried out in (a) fluid droplet(s).

35. The method of claim 34, wherein a plurality of first amplification reactions are carried out in fluid droplets in an emulsion.