~~WHAT IS~~ CLAIM~~ED I~~S:

1. A method of normalizing a nucleic acid library comprising target nucleic acids, comprising:

(a) obtaining a substrate having a normalizing amount of capture probes attached thereto, wherein the capture probes comprise first amplification sites;

(b) hybridizing a plurality of target nucleic acids to the capture probes;

(c) extending the capture probes to obtain extended probes, wherein the extended probes comprise second amplification sites; and

(d) amplifying the extended probes by hybridizing extension primers to the second amplification sites, wherein the amount of the extension primers is equal to or greater than the normalizing amount of capture probes, wherein the amplification is performed such that substantially all the capture probes are extended, thereby obtaining a normalizing amount of amplified target nucleic acids;

wherein the first amplification sites and the extension primers are incapable of or are essentially incapable of hybridizing to one another.

2. The method of claim 1, wherein: ~~the first amplification sites and the extension primers are non-complementary to one another.~~

(i) the first amplification sites and the extension primers:

are non-complementary to one another,

comprise non-complementary nucleotide sequences to one another, or

each lack the same type of nucleotide; or

(ii) the first amplification sites:

comprise modified nucleotides that inhibit hybridization with the extension primers,

lack types of nucleotides complementary to one another and each extension primer consists essentially of the same types of nucleotides as the first amplification site,

lack at least one type of nucleotide selected from adenine (A), cytosine (C), guanine (G), and thymine (T), or

lack a combination of nucleotides selected from: adenine (A) and thymine (T); or guanine (G) and cytosine (C).

~~3. The method of claim 1, wherein the first amplification sites and the extension primers comprise non-complementary nucleotide sequences to one another.~~

~~4. The method of claim 1, wherein the first amplification sites comprise modified nucleotides that inhibit hybridization with the extension primers.~~

~~5. The method of claim 1, wherein the first amplification sites and the extension primers each lack the same type of nucleotide.~~

~~6. The method of claim 1, wherein the first amplification sites lack types of nucleotides complementary to one another, and each extension primer consists essentially of the same types of nucleotides as the first amplification site.~~

~~7. The method of claim 1, wherein the first amplification sites lack at least one type of nucleotide selected from adenine (A), cytosine (C), guanine (G), and thymine (T).~~

~~8. The method of claim 1, wherein the first amplification sites lack a combination of nucleotides selected from: adenine (A) and thymine (T); or guanine (G) and cytosine (C).~~

~~9. The method of claim 7 or 8, wherein the first amplification sites consist of a combination of nucleotides selected from: adenine (A) and guanine (G); adenine (A) and cytosine (C); cytosine (C) and thymine (T); or guanine (G) and thymine (T).~~

~~10. The method of any one of claims 1-9, wherein the first amplification sites consist of a combination of adenine (A) and guanine (G) nucleotides.~~

~~11.~~3. The method of ~~any one of claims 1-10~~claim 1 or 2, wherein the extension primers are in solution.

~~12.~~4. The method of any one of claims 1-~~11~~3, wherein the capture probes comprise first indexes or first sequencing primer sites, and/or first locus specific primers.

~~13. The method of any one of claims 1-12, wherein the capture probes comprise first locus specific primers.~~

~~14.~~5. The method of claim ~~13~~4, wherein the plurality of target nucleic acids hybridize to the first locus specific primers.

~~15.~~6. The method of claim ~~14~~5, wherein extending the capture probes comprises ligating the first locus specific primers to the second locus specific primers.

~~16.~~7. The method of claim ~~15~~6, wherein extending the capture probes comprises:

(i) hybridizing second locus specific primers to the target nucleic acids; and

(ii) ligating the first locus specific primers to the second locus specific primers.

~~17.~~8. The method of claim ~~16~~7, wherein the second locus specific primers comprise the second amplification sites, second indexes, and/or second sequencing primer sites.

~~18. The method of claim 16 or 17, wherein the second locus specific primers comprise second indexes, or second sequencing primer sites.~~

~~19.~~9. The method of claim ~~16~~7, further comprising:

(iii) hybridizing extension oligonucleotides to the second locus specific primers; and

(iv) extending the second locus specific primers with sequences complementary to the extension oligonucleotides by polymerase extension.

~~20.~~10. The method of claim ~~19~~9, wherein the extension oligonucleotides comprise sites complementary to the second amplification sites, complementary to second indexes, or complementary to second sequencing primer sites.

~~21.~~11. The method of any one of claims ~~16-20~~7-10, wherein the target nucleic acids comprise sites complementary to the second amplification sites, and the extending comprises polymerase extension of the first locus specific primers with sequences complementary to the target nucleic acids.

~~22.~~12. The method of claim ~~21~~11, further comprising preparing the target nucleic acids by adding adaptors to an end of the target nucleic acids, wherein the adaptors comprise sites complementary to the second amplification sites.

~~23.~~13. The method of claim ~~22~~12, wherein adding adaptors comprises a tagmentation reaction.

~~24.~~14. The method of any one of claims 1-~~23~~13, wherein the substrate comprises a plurality of beads, and/or a flow cell.

~~25. The method of any one of claims 1-24, wherein the substrate comprises a flow cell.~~

~~26.~~15. The method of any one of claims 1-~~25~~14, wherein the amplification is performed under conditions such that the normalizing amount of capture probes limits the amount of amplification products.

27.16. The method of any one of claims 1-2615, further comprising sequencing the amplified target nucleic acids.

28.17. A method of preparing a library of nucleic acids, comprising:

(a) obtaining a substrate having a plurality of capture probes attached thereto, wherein the capture probes comprise first amplification sites and first locus specific primers;

(b) hybridizing a plurality of target nucleic acids to the first locus specific primers;

(c) hybridizing second locus specific primers to the target nucleic acids;

(d) hybridizing the second locus specific primers to extension oligonucleotides, wherein the extension oligonucleotides comprise sites complementary to second amplification sites; and

(e) extending the hybridized second locus specific primers to obtain a plurality of extended probes by:

(i) ligating the first locus specific primers to the second locus specific primers, and

(ii) extending the ligated second locus specific primers with sequences complementary to the extension oligonucleotides by polymerase extension.

29.18. The method of claim 2817, wherein (i) comprises extending the first locus specific primers, and ligating the first locus specific primers to the second locus specific primers.

30.19. The method of claims 28 or 2917 or 18, wherein the capture probes comprise first indexes, or first sequencing primer sites, and/or the extension oligonucleotides comprise sites complementary to second indexes, or complementary to second sequencing primer sites.

~~31. The method of any one of claims 28-30, wherein the extension oligonucleotides comprise sites complementary to second indexes, or complementary to second sequencing primer sites.~~

~~32. The method of any one of claims 28-31, wherein the substrate comprises a plurality of beads.~~

~~33. The method of any one of claims 28-32, wherein the substrate comprises a flow cell.~~

~~34.~~20. The method of any one of claims ~~28-33~~17-19, further comprising obtaining a normalized amount of amplified target nucleic acids, comprising:

amplifying the extended probes by hybridizing extension primers to the second amplification sites, wherein the substrate comprises a normalizing amount of the capture probes, and the amount of the extension primers is equal to or greater than the normalizing amount of capture probes.

~~35.~~21. The method of claim ~~34~~20, wherein the extension primers are in solution, and/or the amplification is performed under conditions such that the normalizing amount of capture probes limits the amount of amplification products.

~~36. The method of claims 34 or 35, wherein the amplification is performed under conditions such that the normalizing amount of capture probes limits the amount of amplification products.~~

~~37.~~22. The method of ~~any one of claims 34-36~~claim 20 or 21, further comprising sequencing the amplified target nucleic acids.

~~38.~~23. The method of any one of claims ~~28-37~~17-22, wherein the first amplification sites or second amplification sites comprise a P5 sequence, a complement of a P5 sequence, P7 sequence, or a complement of a P7 sequence.

~~39.~~24. The method of any one of claims ~~28-38~~17-23, wherein the first amplification sites and the extension primers are incapable of or are essentially incapable of hybridizing to one another, and wherein:~~.~~

(i) the first amplification sites and the extension primers:

are non-complementary to one another,

comprise non-complementary nucleotide sequences to one another, or

each lack the same type of nucleotide; or

(ii) the first amplification sites:

comprise modified nucleotides that inhibit hybridization with the extension primers,

lack at least one type of nucleotide selected from adenine (A), cytosine (C), guanine (G), and thymine (T), or

lack a combination of nucleotides selected from adenine (A) and thymine (T), or guanine (G) and cytosine (C).

~~40. The method of claim 39, wherein the first amplification sites and the extension primers are non-complementary to one another.~~

~~41. The method of claim 39, wherein the first amplification sites and the extension primers comprise non-complementary nucleotide sequences to one another.~~

~~42. The method of claim 39, wherein the first amplification sites comprise modified nucleotides that inhibit hybridization with the extension primers.~~

~~43. The method of claim 39, wherein the first amplification sites and the extension primers each lack the same type of nucleotide.~~

~~44. The method of claim 39, wherein the first amplification sites lack at least one type of nucleotide selected from adenine (A), cytosine (C), guanine (G), and thymine (T).~~

~~45. The method of claim 39, wherein the first amplification site lacks a combination of nucleotides selected from: adenine (A) and thymine (T); or guanine (G) and cytosine (C).~~

~~46. The method of claim 44 or 45, wherein the first amplification site consists of a combination of nucleotides selected from: adenine (A) and guanine (G); adenine (A) and cytosine (C); cytosine (C) and thymine (T); or guanine (G) and thymine (T).~~

~~47. The method of any one of claims 44-46, wherein the first amplification site consists of a combination of adenine (A) and guanine (G) nucleotides.~~

~~48.~~25. A method of preparing a normalized nucleic acid library, comprising:

(a) obtaining a substrate comprising a plurality of capture probes and a plurality of extension primers attached thereto, wherein the capture probes comprise first amplification sites and first locus specific primers, and the extension primers are capable of hybridizing to second amplification sites, wherein the amount of the capture probes or the extension primers is a normalizing amount;

(b) hybridizing a plurality of target nucleic acids to the first locus specific primers;

(c) extending the capture probes to obtain extended probes, wherein the extended probes comprise second amplification sites;

(e) amplifying the extended probes by hybridizing the extended probes to the extension primers, to obtain a normalized amount of amplified target nucleic acids.

~~49.~~26. The method of claim ~~48~~25, wherein the normalizing amount limits the amount of amplified target nucleic acids.

~~50.~~27. The method of claim ~~48 or 49~~25 or 26, wherein the capture probes comprise first indexes, or first sequencing primer sites; and/or the extension oligonucleotides comprise sites complementary to second indexes, or complementary to second sequencing primer sites.

~~51. The method of any one of claims 48-50, wherein the extension oligonucleotides comprise sites complementary to second indexes, or complementary to second sequencing primer sites.~~

~~52.~~28. The method of any one of claims ~~48-51~~25-27, wherein the amplification is performed under conditions such that the normalizing amount of capture probes or the extension primers limits the amount of amplification products.

~~53. The method of any one of claims 48-52, wherein the substrate comprises a plurality of beads.~~

~~54. The method of any one of claims 48-53, wherein the substrate comprises a flow cell.~~

~~55.~~29. The method of any one of claims ~~48-54~~25-28, wherein the amplification comprises bridge amplification.

~~56. The method of any one of claims 48-55, further comprising sequencing the amplified target nucleic acids.~~

~~57.~~30. The method of claim ~~48~~25, wherein: ~~extending the capture probes comprises ligating the first locus specific primers to the second locus specific primers.~~

(i) extending the capture probes comprises ligating the first locus specific primers to the second locus specific primers;

(ii) extending the capture probes comprises hybridizing second locus specific primers to the target nucleic acids, and ligating the first locus specific primers to the second locus specific primers hybridized to the target nucleic acids; or

(iii) the method further comprises: hybridizing extension oligonucleotides to the second locus specific primers; and extending the second locus specific primers with sequences complementary to the extension oligonucleotides by polymerase extension.

~~58. The method of claim 48, wherein extending the capture probes comprises:~~

~~(i) hybridizing second locus specific primers to the target nucleic acids; and~~

~~(ii) ligating the first locus specific primers to the second locus specific primers.~~

~~59.~~31. The method of claim ~~58~~30, wherein the second locus specific primers comprise the second amplification sites, second indexes and/or second sequencing primer sites.

~~60. The method of claim 58 or 59, wherein the second locus specific primers comprise second indexes or second sequencing primer sites.~~

~~61. The method of claim 48, further comprising:~~

~~(iii) hybridizing extension oligonucleotides to the second locus specific primers; and~~

~~(iv) extending the second locus specific primers with sequences complementary to the extension oligonucleotides by polymerase extension.~~

~~62.~~32. The method of claim ~~61~~30, wherein the extension oligonucleotides comprise sites complementary to the second amplification sites, complementary to second indexes, or complementary to second sequencing primer sites; and/or wherein the target nucleic acids comprise sites complementary to the second amplification sites, and the extending comprises polymerase extension of the first locus specific primers with sequences complementary to the target nucleic acids.

~~63. The method of claim 61 or 62, wherein the target nucleic acids comprise sites complementary to the second amplification sites, and the extending comprises polymerase extension of the first locus specific primers with sequences complementary to the target nucleic acids.~~

~~64.~~33. The method of claim ~~63~~30 or 32, further comprising preparing the target nucleic acids by adding adaptors to an end of the target nucleic acids, wherein the adaptors comprise sites complementary to the second amplification sites.

~~65.~~34. The method of claim ~~64~~33, wherein adding adaptors comprises a tagmentation reaction.

~~66. The method of any one of claims 48-65, further comprising determining the presence or absence of a variant in the target nucleic acid.~~

~~67.~~35. A method of preparing an indexed nucleic acid library, comprising:

(a) adding Y-adaptors to a plurality of target nucleic acids, wherein the Y-adaptors are added to first and second ends of each target nucleic acid, and each Y-adaptor comprises a first strand comprising a first primer binding site and a mosaic

element, and a second strand comprising a second primer binding site and a complement to the mosaic element; and

(b) hybridizing a first extension oligonucleotide to the second primer binding site, wherein the first extension oligonucleotide comprises a first index and a third primer binding site; and

(c) extending the second strand comprising the second primer binding site, thereby adding the first index to the target nucleic acids.

~~68.~~36. The method of claims ~~67~~35, wherein a 5′ end of the first strand of the Y-adaptor is resistant to nuclease degradation; a 5′ end of the first strand of the Y-adaptor comprises a phosphorothioate bond between two consecutive nucleotides; and/or a 5′ end of the second strand of the Y-adaptor is phosphorylated.

~~69. The method of claims 67 or 68, wherein a 5′ end of the first strand of the Y-adaptor comprises a phosphorothioate bond between two consecutive nucleotides.~~

~~70. The method of any one of claims 67-69, wherein a 5′ end of the second strand of the Y-adaptor is phosphorylated.~~

~~71.~~37. The method of ~~any one of claims 67-70~~claim 35 or 36, wherein (a) comprises contacting the plurality of target nucleic acids with a plurality of transposomes, wherein each transposome comprises a Y-adaptor and a transposase.

~~72.~~38. The method of claim ~~71~~37, wherein the transposomes comprise dimers; the transposase comprises a Tn5 transposase; and/or the transposomes are bound to a substrate.

~~73. The method of claim 71 or 72, wherein the transposase comprises a Tn5 transposase.~~

~~74. The method of claim 71-73, wherein the transposomes are bound to a substrate.~~

~~75. The method of claim 74, wherein the substrate comprises a plurality of beads.~~

~~76. The method of claim 75, wherein the beads are magnetic.~~

~~77.~~39. The method of any one of claims ~~67-76~~35-38, wherein a 3′ end of the first extension oligonucleotide is blocked, and/or a 5′ end of the first extension oligonucleotide is phosphorylated.

~~78. The method of any one of claims 67-77, wherein a 5′ end of the first extension oligonucleotide is phosphorylated.~~

~~79.~~40. The method of any one of claims ~~67-78~~35-39, wherein the first extension oligonucleotide is bound to a bead, the method further comprising cleaving the first extension oligonucleotide from the bead prior to (c).

~~80. The method of claim 79, further comprising cleaving the first extension oligonucleotide from the bead prior to (c).~~

~~81.~~41. The method of any one of claims ~~67-80~~35-40, wherein (c) comprises polymerase extension; or extension with a ligase comprising:~~.~~

hybridizing a ligation oligonucleotide to the first extension oligonucleotide hybridized to the second primer binding site, and

ligating the ligation oligonucleotide to the second strand comprising the second primer binding site.

~~82. The method of any one of claims 67-80, wherein (c) comprises extension with a ligase.~~

~~83. The method of claim 82, wherein extension with a ligase comprises:~~

~~hybridizing a ligation oligonucleotide to the first extension oligonucleotide hybridized to the second primer binding site; and~~

~~ligating the ligation oligonucleotide to the second strand comprising the second primer binding site.~~

~~84.~~42. The method of claim ~~83~~41, wherein the ligation oligonucleotide comprises an additional index, or an additional primer binding site.

~~85. The method of claim 83, wherein the ligation oligonucleotide comprises an additional primer binding site.~~

~~86.~~43. The method of any one of claims ~~67-85~~35-42, further comprising removing the first extension oligonucleotide after step (c); and optionally, wherein the removing comprises an exonuclease treatment.

~~87. The method of claim 86, wherein the removing comprises an exonuclease treatment.~~

~~88. The method of claim 87, wherein the first extension oligonucleotide comprises uracil nucleotides, and the removing comprises degradation of the first extension oligonucleotide with a uracil-specific excision reagent (USER) enzyme.~~

89.44. The method of any one of claims 67-8835-43, further comprising amplifying the target nucleic acids comprising the first index, wherein the amplification comprises hybridizing amplification primers to the first primer binding sites and to the third primer binding sites; and/or the amplification comprises a bridge amplification.

90. The method of claim 89, wherein the amplification comprises hybridizing amplification primers to the first primer binding sites and to the third primer binding sites.

91. The method of claim 89 or 90, wherein the amplification comprises a PCR.

92. The method of claim 89 or 90, wherein the amplification comprises bridge amplification.

93.45. The method of any one of claims 67-9235-44, further comprising adding a second index to the target nucleic acids comprising the first index, comprising:.

hybridizing a second extension oligonucleotide to the third primer binding site of the target nucleic acids comprising the first index, wherein the second extension oligonucleotide comprises the second index; and

extending the second strand comprising the third primer binding site, thereby adding the second index to the target nucleic acids comprising the first index.

94. The method of claim 93, wherein adding the second index comprises:

hybridizing a second extension oligonucleotide to the third primer binding site of the target nucleic acids comprising the first index, wherein the second extension oligonucleotide comprises the second index; and

extending the second strand comprising the third primer binding site, thereby adding the second index to the target nucleic acids comprising the first index.

95.46. The method of claim 9445, wherein a 3′ end of the second extension oligonucleotide is blocked, and/or a 5′ end of the second extension oligonucleotide is phosphorylated.

96. The method of claim 94 or 95, wherein a 5′ end of the second extension oligonucleotide is phosphorylated.

97.47. The method of any one of claims 94-96claim 45 or 46, wherein: extending the second strand comprising the third primer binding site comprises polymerase extension, or extension with a ligase; the method further comprises removing the second extension

oligonucleotide after extending the third primer binding site; and/or the method further comprises adding a third index to the target nucleic acids comprising the second index.

~~98. The method of any one of claims 94-96, wherein extending the second strand comprising the third primer binding site comprises extension with a ligase.~~

~~99. The method of any one of claims 94-98, further comprising removing the second extension oligonucleotide after extending the third primer binding site.~~

~~100. The method of any one of claims 94-99, further comprising adding a third index to the target nucleic acids comprising the second index.~~

~~101. The method of claim 100, further comprising adding an additional index to the target nucleic acids comprising the third index.~~

~~102. The method of any one of claims 67-101, wherein the target nucleic acids comprise genomic DNA.~~

~~103.~~48. A method of combinatorial indexing a plurality of target nucleic acids, comprising:

(a) obtaining a pool of primary indexed nucleic acids, comprising:

adding a first index to a plurality of subpopulations of target nucleic acids by extending the target nucleic acids with the first index, wherein a different first index is added to each subpopulation, and

combining the subpopulations comprising the different first indexes to obtain the pool of primary indexed nucleic acids;

(b) splitting the pool into a plurality of subpopulations of primary indexed nucleic acids;

(c) obtaining a pool of secondary indexed nucleic acids, comprising:

adding a second index to the plurality of subpopulations of primary indexed nucleic acids by extending the primary indexed nucleic acids with the second index, wherein a different second index is added to each subpopulation, and

combining the subpopulations comprising the different second indexes to obtain the pool of secondary indexed nucleic acids

~~104.~~49. The method of claim ~~103~~48, further comprising repeating (b) and (c) and adding additional indexes to indexed subpopulations; and/or wherein adding a first index

to a subpopulation of target nucleic acids is performed in a compartment selected from a well, a channel, or a droplet.

~~105. The method of claim 103 or 104, wherein adding a first index to a subpopulation of target nucleic acids is performed in a compartment selected from a well, a channel, or a droplet.~~

~~106. The method of any one of claims 1-106, wherein the amplifying and/or the extending is performed under conditions suitable to remove inorganic pyrophosphate (PP_i), to inhibit pyrohosphorolysis, and/or to inhibit formation of a Mg^{2+}-PPi complex.~~

~~107.~~50. A method of modifying a nucleic acid comprising:

amplifying or extending the nucleic acid in the presence of a polymerase, wherein the amplifying and/or the extending is performed under conditions suitable to remove inorganic pyrophosphate (PP_i), to inhibit pyrohosphorolysis, and/or to inhibit formation of a Mg^{2+}-PPi complex; and optionally, wherein the PPi is soluble.

~~108. The method of claim 106 or 107, wherein the PPi is soluble.~~

~~109. The method of any one of claims 1-108, wherein the amplifying and/or the extending is performed in the presence of an inorganic pyrophosphatase.~~

~~110. The method of claim 109, wherein a rate of achieving a yield of a product of the amplifying and/or the extending is increased in the presence of the inorganic pyrophosphatase compared to a rate of achieving a yield of a product of the amplifying and/or the extending in the absence of the inorganic pyrophosphatase.~~

~~111. The method of claim 110, wherein the rate of achieving a yield of a product of the amplifying and/or the extending is increased by at least 2-fold.~~

~~112. The method of any one of claims 1-111, wherein the amplifying and/or the extending is performed under isothermal conditions.~~

~~113. The method of any one of claims 1-112, wherein the amplifying and/or the extending comprises performing a reaction selected from a PCR, a bridge amplification, a whole genome amplification, a loop-mediated isothermal amplification (LAMP), an amplification from nucleic acids obtained from a single cell, a sequencing by synthesis (SBS) reaction, and an exclusion amplification (ExAMp).~~

~~114. The method of any one of claims 1-113, wherein the amplifying and/or the extending comprises performing an amplification from nucleic acids obtained from a single cell.~~

~~115. The method of any one of claims 1-113, wherein the amplifying and/or the extending comprises performing a sequencing by synthesis (SBS) reaction.~~

~~116. The method of any one of claims 1-113, wherein the amplifying and/or the extending comprises performing a bridge amplification.~~

~~117. The method of any one of claims 1-113, wherein a sequencing reaction comprises the amplifying and/or the extending.~~