CLAIMS:

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:

(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 or 2, wherein the extension primers are in solution.

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

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

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

7. The method of claim 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.

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

9. The method of claim 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.

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

11. The method of any one of claims 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.

12. The method of claim 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.

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

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

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

16. The method of any one of claims 1-15, further comprising sequencing the amplified target nucleic acids.

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.

18. The method of claim 17, wherein (i) comprises extending the first locus specific primers, and ligating the first locus specific primers to the second locus specific primers.

19. The method of claims 17 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.

20. The method of any one of claims 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.

21. The method of claim 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.

22. The method of claim 20 or 21, further comprising sequencing the amplified target nucleic acids.

23. The method of any one of claims 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.

24. The method of any one of claims 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).

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.

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

27. The method of claim 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.

28. The method of any one of claims 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.

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

30. The method of claim 25, wherein:

(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.

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

32. The method of claim 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.

33. The method of claim 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.

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

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.

36. The method of claims 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.

37. The method of 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.

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

39. The method of any one of claims 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.

40. The method of any one of claims 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).

41. The method of any one of claims 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.

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

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

44. The method of any one of claims 35-43, 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.

45. The method of any one of claims 35-44, 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.

46. The method of claim 45, wherein a 3′ end of the second extension oligonucleotide is blocked, and/or a 5′ end of the second extension oligonucleotide is phosphorylated.

47. The method of claim 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.

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

49. The method of claim 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.

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.