

CLAIMS:

1. A method for synthesizing a nucleic acid, comprising:
 - providing a plurality of nucleic acid fragments having overlapping sequences, wherein the plurality of nucleic acid fragments have complementary sequences to at least one other fragment of the plurality of nucleic acid fragments, wherein a first fragment of the plurality of nucleic acid fragments comprises a first cleavable adapter sequence and a 5' end of the target sequence downstream of the first cleavable adapter sequence and wherein a last fragment of the plurality of nucleic acid fragments comprises a 3' end of the target sequence upstream of a second cleavable adapter sequence;
 - immobilizing the first fragment on a substrate with a first immobilized primer complementary to the first cleavable adapter sequence;
 - assembling the plurality of nucleic acid fragments into an assembled polynucleotide molecule via hybridization of the complementary sequences, wherein the assembled polynucleotide molecule is immobilized on the substrate via the first immobilized primer;
 - amplifying the assembled polynucleotide molecule on the substrate to generate an amplified cluster comprising amplicons of the assembled polynucleotide molecule on the substrate; and
 - sequencing the amplified cluster.
2. The method of claim 1, wherein the providing of the plurality of nucleic acid fragments comprises in vitro synthesizing the nucleic acid fragments.
3. The method of claim 2, wherein the plurality of nucleic acid fragments comprises known portions of a target sequence.
4. The method of claim 1, wherein an assembled double-stranded polynucleotide is formed by the assembling of the plurality of nucleic acid fragments, and wherein the method further comprises a step of denaturing the assembled double-stranded polynucleotide molecule to generate a single-stranded polynucleotide molecule that extends from the first immobilized primer.

5. The method of claim 1, wherein the assembled polynucleotide molecule, that is formed by the assembling of the plurality of nucleic acid fragments, is partially double stranded, whereby the assembled polynucleotide molecule comprises gaps in at least one strand.

6. The method of claim 5, wherein the method further comprises a step of filling the gaps in a strand of the assembled polynucleotide molecule by polymerase extension.

7. The method of claim 1, wherein the amplifying comprises bridge amplification of the assembled polynucleotide molecule on the substrate.

8. The method of claim 1, wherein the plurality of nucleic acid fragments is single stranded nucleic acids.

9. The method of claim 1, comprising harvesting the amplified cluster if a sequence of the cluster includes a target sequence or is complementary to the target sequence.

10. The method of claim 9, wherein the amplified cluster includes the target sequence or is complementary to the target sequence if the cluster sequence has no errors.

11. The method of claim 9, wherein the amplified cluster includes the target sequence or is complementary to the target sequence if the cluster sequence has a sequence error rate less than a predetermined threshold.

12. The method of claim 9, wherein harvesting the amplified cluster comprises cleaving the second cleavable adapter and/or the first cleavable adapter.

13. The method of claim 9, wherein harvesting the amplified cluster comprises cleaving the second cleavable adapter and/or the first cleavable adapter optically, chemically, magnetically, electrically, electromagnetically, or any combination thereof.

14. The method of claim 1, comprising generating a sequence accuracy score or metric for each of the plurality of amplified clusters.

15. The method of claim 1, wherein each of the plurality of nucleic acid fragments is less than 200 nucleotides in length.

16. The method of claim 1, wherein the overlapping sequences of the plurality of nucleic acid fragments are between 20 and 30 nucleotides long.

17. The method of claim 1, wherein the assembling comprises assembling the plurality of nucleic acid fragments in the presence of DNA recombinase, beta protein, DNA polymerase, DNA ligase, circ ligase, or any combination thereof.

18. The method of claim 1, wherein the substrate comprises a bead, a magnetic bead, a glass slide, a microchip, a nano droplet, an electrowetting cartridge, or any combination thereof.

19. A method for synthesizing a plurality of nucleic acids, comprising carrying out the method of claim 1 to immobilize a plurality of different first fragments on the substrate such that a plurality of different single-stranded fragments are assembled, a plurality of different amplified clusters are generated and a plurality of different amplified clusters are sequenced.

20. The method of claim 19, comprising selectively harvesting a target amplified cluster compared to other amplified clusters in the plurality of different amplified clusters, wherein the target amplified cluster has highest sequence accuracy relative to the other amplified clusters.

21. The method of claim 19, comprising selectively harvesting a target amplified cluster compared to other amplified clusters in the plurality of different amplified clusters, wherein the target amplified cluster has low sequence accuracy relative to the other amplified clusters.

22. The method of claim 19, comprising generating a sequence accuracy score or metric for each of the plurality of amplified clusters.

23. A method for synthesizing nucleic acid fragments, comprising:
providing a plurality of oligonucleotides immobilized on a substrate via hybridization to a plurality of first immobilized primers complementary to the 5' ends of the oligonucleotides, wherein each respective oligonucleotide comprises a first adapter sequence at a 5' end;
extending the oligonucleotides to generate extended polynucleotides corresponding to a fragment sequence, wherein the extending comprises incorporating a plurality of individual nucleotides or nucleic acids onto a 3' end of the respective oligonucleotides of the plurality of oligonucleotides to generate the extended polynucleotides having the fragment sequence;
attaching a second adapter sequence at the 3' ends of the extended polynucleotides having the fragment sequence;
annealing the 3' ends of the extended polynucleotides to the substrate via a plurality of second immobilized primers complementary to the 3' ends of the extended polynucleotides to form bridges;
amplifying the bridges to form a plurality of amplified clusters;
sequencing the plurality of amplified clusters to determine if one or more of the plurality of amplified clusters includes a sequence of the nucleic acid fragment;
harvesting the one or more of the plurality of amplified clusters if a sequence of the one or more of the plurality of amplified clusters includes the sequence of the nucleic acid fragment or is complementary to the sequence of the nucleic acid fragment to generate harvested amplified clusters having the fragment sequence; and
pooling the harvested amplified clusters having the fragment sequence with a second plurality of amplified clusters, wherein at least a portion of the second plurality of amplified clusters comprises a sequence complementary to only a portion of the fragment sequence.

24. The method of claim 23, wherein the extending is carried out by a first polymerase that incorporates the plurality of individual nucleotides onto the 3' end of the respective oligonucleotides of the plurality of oligonucleotides to generate the extended polynucleotides having the fragment sequence.

25. The method of claim 23, wherein the extending is carried out by a ligase that incorporates the plurality of individual nucleic acids onto the 3' end of the respective

oligonucleotides of the plurality of oligonucleotides to generate the extended polynucleotides having the fragment sequence.

26. The method of claim 23, wherein the ligase comprises circ ligase.

27. The method of claim 23, wherein the second plurality of amplified clusters comprises a second fragment sequence, wherein the second fragment sequence comprises the sequence complementary to only a portion of the fragment sequence.

28. The method of claim 23, wherein the one or more of the plurality of amplified clusters includes the sequence of the nucleic acid fragment or is complementary to the sequence of the nucleic acid fragment if the sequence of the one or more of the plurality of amplified clusters has a sequence error rate less than a predetermined threshold.

29. The method of claim 23, wherein harvesting the one or more of the plurality of amplified clusters comprises cleaving the second adapter sequence and/or the first adapter sequence.

30. The method of claim 23, wherein harvesting the one or more of the plurality of amplified clusters comprises cleaving the second adapter sequence and/or the first adapter sequence optically, chemically, magnetically, electrically, electromagnetically, or any combination thereof.

31. The method of claim 23, comprising harvesting one of the plurality of amplified clusters having a highest sequence accuracy relative to other clusters in the plurality of amplified clusters.

32. The method of claim 23, comprising generating a sequence accuracy score or metric for each of the plurality of amplified clusters.

33. The method of claim 24, wherein the first polymerase comprises terminal deoxynucleotidyl transferase (TdT).

34. The method of claim 23, wherein the substrate comprises a bead, a magnetic bead, a glass slide, a microchip, a nano droplet, an electrowetting cartridge, or any combination thereof.

35. A method for synthesizing a nucleic acid, comprising:

providing a plurality of target sequences based on a sequence of the nucleic acid, wherein a combination of the plurality of target sequences form the sequence of the nucleic acid;

providing a plurality of primer oligonucleotides immobilized on a substrate;

extending the primer oligonucleotides in a single-stranded manner based on the plurality of target sequences in the presence of a first polymerase to generate a plurality of fragment polynucleotides;

providing a first sequencing reagent to the plurality of fragment polynucleotides in the presence of a second polymerase, wherein the first sequencing reagent comprises one or more nucleotide monomers, and wherein the one or more nucleotide monomers form a plurality of polynucleotides complementary to at least a portion of the plurality of fragment polynucleotides;

providing a second sequencing reagent to the plurality of fragment polynucleotides, wherein the second sequencing reagent comprises at least one nucleotide monomer, wherein the at least one nucleotide monomer of the second sequencing reagent comprises a reversibly terminating moiety, and wherein the second sequencing reagent is provided subsequent to providing the first sequencing reagent, whereby a sequence of each of the plurality of fragment polynucleotides is obtained;

harvesting the plurality of fragment polynucleotides based on comparing the sequence of each of the plurality of fragment polynucleotides with the respective target sequence; and

assembling the plurality of fragment polynucleotides to generate an assembled polynucleotide.

36. The method of claim 35, comprising providing a plurality of amplification oligonucleotides immobilized on the substrate, and amplifying the plurality of fragment polynucleotides, wherein the amplifying comprises annealing one or more of the plurality of immobilized amplification oligonucleotides to one or more of the plurality of fragment polynucleotides.

37. The method of claim 35, comprising amplifying the assembled polynucleotide, wherein the amplifying comprises annealing one or more of the plurality of immobilized amplification oligonucleotides to one or more of the assembled polynucleotide.

38. The method of claim 35, comprising:

providing a third sequencing reagent to the assembled polynucleotide in the presence of a third polymerase, wherein the third sequencing reagent comprises one or more nucleotide monomers, and wherein the one or more nucleotide monomers form a plurality of polynucleotides complementary to at least a portion of the assembled polynucleotide; and

providing a fourth sequencing reagent to the assembled polynucleotide, wherein the fourth sequencing reagent comprises at least one nucleotide monomer, wherein the at least one nucleotide monomer of the fourth sequencing reagent comprises a reversibly terminating moiety, and wherein the fourth sequencing reagent is provided subsequent to providing the third sequencing reagent, whereby sequence information for the assembled polynucleotide is obtained.

39. The method of claim 35, wherein harvesting the plurality of fragment polynucleotides if the sequence of each of the plurality of fragment polynucleotides includes the respective target sequence or is complementary to the respective target sequence.

40. The method of claim 36, wherein the sequence of each of the plurality of fragment polynucleotides includes the respective target sequence or is complementary to the respective target sequence if the sequence of each of the plurality of fragment polynucleotides has a sequence error rate less than a predetermined threshold.

41. The method of claim 35, wherein harvesting the plurality of fragment polynucleotides comprises cleaving the plurality of fragment polynucleotides from the plurality of primer oligonucleotides.

42. The method of claim 35, comprising generating a sequence accuracy score or metric for each of the plurality of fragment polynucleotides.

43. The method of claim 35, wherein the first polymerase comprises terminal deoxynucleotidyl transferase (TdT).

44. The method of claim 35, wherein the substrate comprises a bead, a magnetic bead, a glass slide, a microchip, a nano droplet, an electrowetting cartridge, or any combination thereof.

45. A system for synthesizing a nucleic acid, comprising:
a substrate comprising a plurality of flow channels, each of the plurality of flow channels comprising a plurality of immobilized primer oligonucleotides;
a processor-based device storing executable instructions and coupled to the substrate, wherein the executable instructions are configured to:

receive one or more synthesis signals for each respective flow channel indicative of a presence of a first polymerase generating a respective plurality of single-stranded fragment polynucleotides based on one of a plurality of target sequences;

control entry of a first sequencing reagent, a second sequencing reagent, and a second polymerase into the flow channels based on the one or more synthesis signals, wherein the first sequencing reagent comprises one or more nucleotide monomers, wherein the one or more nucleotide monomers form a plurality of polynucleotides complementary to at least a portion of the plurality of fragment polynucleotides, wherein the second sequencing reagent comprises at least one nucleotide monomer, and wherein the at least one nucleotide monomer of the second sequencing reagent comprises a reversibly terminating moiety;

receive one or more sequencing signals for each respective flow channel indicative of a presence of the first sequencing reagent and the second sequencing reagent in the presence of the second polymerase;

determine a sequence of each of the plurality of fragment polynucleotides based on the one or more sequencing signals; and

provide an indication related to a comparison of the sequence of each of the plurality of fragment polynucleotides with the respective target sequence; and

an assembly cell configured to gather each of the plurality of fragment polynucleotides from each of the plurality of flow channels and assemble each of the plurality of fragment polynucleotides to generate an assembled polynucleotide.

46. The system of claim 45, wherein each of the plurality of flow channels is configured to immobilize a plurality of amplification oligonucleotides on the substrate, and wherein the executable instructions further comprise instructions to amplify the plurality of fragment polynucleotides by annealing the fragment polynucleotides to one or more of the plurality of immobilized amplification oligonucleotides.

47. The system of claim 45, wherein the executable instructions further comprise instructions to amplify the assembled polynucleotide by annealing to one or more of the plurality of immobilized amplification oligonucleotides in the assembly cell.

48. The system of claim 45, wherein the executable instructions further comprise instructions to receive a third sequencing reagent at the assembly cell in the presence of a third polymerase and to receive a fourth sequencing reagent, wherein the third sequencing reagent comprises one or more nucleotide monomers, wherein the one or more nucleotide monomers form a plurality of polynucleotides complementary to at least a portion of the assembled polynucleotide, wherein the fourth sequencing reagent comprises at least one nucleotide monomer, wherein the at least one nucleotide monomer of the fourth sequencing reagent comprises a reversibly terminating moiety, and wherein the fourth sequencing reagent is provided subsequent to providing the third sequencing reagent, whereby sequence information for the assembled polynucleotide is obtained.

49. The system of claim 45, wherein the executable instructions further comprise instructions to harvest the plurality of fragment polynucleotides if the sequence of each of the plurality of fragment polynucleotides is identified to include the respective target sequence or to be complementary to the respective target sequence.

50. The system of claim 49, wherein the executable instructions further comprise instructions to harvest the plurality of fragment polynucleotides if the sequence of each of

the plurality of fragment polynucleotides has a sequence error rate less than a predetermined threshold.

51. The system of claim 45, wherein the executable instructions further comprise instructions to harvest the plurality of fragment polynucleotides by cleaving the plurality of fragment polynucleotides from the plurality of primer oligonucleotides.

52. The system of claim 45, comprising a processor configured to generate a sequence accuracy score or metric for each of the plurality of fragment polynucleotides.

53. The system of claim 45, wherein the first polymerase comprises terminal deoxynucleotidyl transferase (TdT).

54. The system of claim 45, wherein the substrate comprises a bead, a magnetic bead, a glass slide, a microchip, a nano droplet, an electrowetting cartridge, or any combination thereof.

55. A method for synthesizing a nucleic acid, comprising:
providing a starting polynucleotide sequence including a target sequence;
receiving or accessing information representative of sequences of a plurality of overlapping nucleic acid fragments based on the target sequence, wherein the fragments are designed such that the fragments including the target sequence comprise discontinuities in a 5' to 3' direction, and wherein the discontinuities between nucleic acid fragments occur when the nucleic acid fragments are hybridized on a complementary strand to the target sequence;
receiving sequencing information from amplified clusters, wherein the amplified clusters are amplified from bridges generated from synthesized fragments having the sequence of the plurality of nucleic acid fragments; and
determining a quality of one or more of the plurality of amplified clusters based on the sequencing information.

56. The method of claim 55, wherein the discontinuities comprise gaps, whereby nucleic acid fragments are separated by the space of at least one nucleotide when hybridized to the complementary strand.

57. The method of claim 55, wherein the discontinuities comprise nicks, whereby nucleic acid fragments are juxtaposed at the nick when hybridized to the complementary strand.

58. The method of claim 55, comprising redesigning the plurality of nucleic acid fragments if the amplified clusters have an error rate for the target sequence that is greater than a predetermined threshold.

59. The method of claim 58, wherein redesigning the plurality of nucleic acid fragments comprises designing sequences with different areas of discontinuity.

60. The method of claim 55, comprising receiving information about a yield of each of the synthesized fragments of the plurality of nucleic acid fragments and redesigning the plurality of single-stranded fragments if a yield of one or more of the synthesized fragments is below a predetermined threshold.

61. The method of claim 55, comprising selectively harvesting an amplified cluster if a sequence of the cluster includes a target sequence or is complementary to the target sequence.

CLAIMS:

1. A method for synthesizing a nucleic acid, comprising:
 - providing a target sequence of a nucleic acid comprising a first end sequence, a second end sequence, and an intermediate sequence therebetween;
 - providing a plurality of overlapping nucleic acid fragments, wherein the plurality of nucleic acid fragments have complementary portions to at least one other fragment of the plurality of nucleic acid fragments, wherein a first fragment of the plurality of nucleic acid fragments comprises a first cleavable adapter and a portion having the first end sequence, wherein an end of the first fragment comprises the first cleavable adapter, and wherein a last fragment of the plurality of nucleic acid fragments comprises a second cleavable adapter, and a portion having the second end sequence, wherein an end of the last fragment comprises the second cleavable adapter, and comprising: generating the first fragment by *de novo* extension of an oligonucleotide immobilized on a synthesis substrate, and selectively harvesting the first fragment from the synthesis substrate;
 - immobilizing the first fragment on an assembly substrate with a first immobilized primer complementary to the first cleavable adapter;
 - assembling the plurality of nucleic acid fragments into an assembled polynucleotide molecule via hybridization of the complementary portions, wherein the assembled polynucleotide molecule is partially double stranded and comprises gaps in both strands, wherein the assembled polynucleotide molecule is immobilized on the assembly substrate via the first immobilized primer;
 - amplifying the assembled polynucleotide molecule on the assembly substrate to generate an amplified cluster comprising amplicons of the assembled polynucleotide molecule on the assembly substrate; and
 - sequencing the amplified cluster.
2. The method of claim 1, wherein the assembling further comprises a step of filling the gaps in a strand of the assembled polynucleotide molecule by polymerase extension.
3. The method of claim 2, wherein an assembled double-stranded polynucleotide is formed by the assembling of the plurality of nucleic acid fragments, and wherein the method

further comprises a step of denaturing the assembled double-stranded polynucleotide molecule to generate a single-stranded polynucleotide molecule that extends from the first immobilized primer.

4. The method of claim 1, wherein the amplifying comprises bridge amplification of the assembled polynucleotide molecule on the assembly substrate.

5. The method of claim 1, wherein the plurality of nucleic acid fragments is single stranded nucleic acids.

6. The method of claim 1, comprising selectively harvesting the amplified cluster, wherein a sequence of the amplified cluster is determined to have a sequence error rate compared to the target sequence or a complement of the target sequence less than or equal to a predetermined threshold.

7. The method of claim 6, wherein the predetermined threshold is zero.

8. The method of claim 6, wherein selectively harvesting the amplified cluster comprises cleaving the second cleavable adapter and/or the first cleavable adapter.

9. The method of claim 6, wherein selectively harvesting the amplified cluster comprises cleaving the second cleavable adapter and/or the first cleavable adapter optically, chemically, magnetically, electrically, electromagnetically, or any combination thereof.

10. The method of claim 1, comprising comparing a sequence of the amplified cluster with the target sequence or a complement of the target sequence to generate a sequence accuracy score or sequence error rate for the amplified cluster.

11. The method of claim 1, wherein each of the plurality of nucleic acid fragments is less than 200 nucleotides in length.

12. The method of claim 1, wherein the overlapping portions of the plurality of nucleic acid fragments are between 20 and 30 nucleotides long.

13. The method of claim 1, wherein the assembling comprises assembling the plurality of nucleic acid fragments in the presence of DNA recombinase, DNA polymerase, DNA ligase, or any combination thereof.

14. The method of claim 1, wherein the substrate comprises a bead, a magnetic bead, a glass slide, a microchip, a nano droplet, an electrowetting cartridge, or any combination thereof.

15. A method for synthesizing a plurality of nucleic acids, comprising carrying out the method of claim 1 to immobilize a plurality of different first fragments on the substrate such that

a plurality of different single-stranded fragments are assembled, a plurality of different amplified clusters are generated and a plurality of different amplified clusters are sequenced.

16. The method of claim 15, comprising selectively harvesting a target amplified cluster compared to other amplified clusters in the plurality of different amplified clusters, wherein the target amplified cluster has highest sequence error rate relative to the other amplified clusters, wherein the sequence error rate is determined by comparing a sequence of each amplified cluster with the target sequence or a complement of the target sequence.

17. The method of claim 15, comprising selectively harvesting a target amplified cluster compared to other amplified clusters in the plurality of different amplified clusters, wherein the target amplified cluster has lowest sequence error rate relative to the other amplified clusters, wherein the sequence error rate is determined by comparing a sequence of each amplified cluster with the target sequence or a complement of the target sequence.

18. The method of claim 15, comprising comparing a sequence of each amplified cluster with the target sequence or a complement of the target sequence to generate a sequence accuracy score or sequence error rate for each of the plurality of amplified clusters.