CLAIMS

WHAT WE CLAIM IS:

1. A method for nucleic acid assembly, comprising:

(a) providing a first plurality of polynucleotides, comprising up to 2500 bases in length, wherein each polynucleotide of the first plurality of polynucleotides comprises a first terminal region of sequence homology;

(b) providing a second plurality of polynucleotides, comprising up to 2500 bases in length, wherein each polynucleotide of the second plurality of polynucleotides comprises a second terminal region of sequence homology to the first terminal region of sequence homology; and

(c) contacting the first plurality of polynucleotides and the second plurality of polynucleotides with a reaction mixture comprising an exonuclease, a~~n~~ flap endonuclease 1, a polymerase, and a ligase to assemble a library of nucleic acids, wherein at least 80% of the nucleic acids are each present in the library in an amount within 2x of a mean frequency for each of the nucleic acids in the library.

2. The method of claim 1, wherein the first plurality of polynucleotides comprises up to 100 different sequences, and wherein the second plurality of polynucleotides comprises up to 100 different sequences.

~~3. The method of claim 1, wherein the second plurality of polynucleotides comprises up to 100 different sequences.~~

~~4.~~3. The method of claim 1, wherein at least 10,000 nucleic acids are assembled.

~~5.~~4. The method of claim 1, wherein at least 100,000 nucleic acids are assembled.

~~6. The method of claim 1, wherein each polynucleotide of the first plurality of polynucleotides comprises up to 2500 bases in length.~~

~~7. The method of claim 1, wherein each polynucleotide of the second plurality of polynucleotides comprises up to 2500 bases in length.~~

~~8. The method of claim 1, wherein the exonuclease is exonuclease III.~~

~~9. The method of claim 1, wherein the endonuclease is a flap endonuclease.~~

~~10. The method of claim 9, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.~~

~~11.~~5. The method of claim 1, wherein the polymerase comprises 5’ to 3’ polymerase activity.

~~12.~~6. The method of claim 1, wherein the polymerase is a DNA polymerase.

~~13.~~7. The method of claim 1, wherein the ligase catalyzes joining of at least two nucleic acids.

~~14.~~8. A method for nucleic acid assembly, comprising:

(a) de novo synthesizing a first nucleic acid comprising in 5’ to 3’ order: a barcode sequence, a first restriction endonuclease site, a second restriction endonuclease site, and a first hypervariable region sequence;

(b) de novo synthesizing a second nucleic acid comprising in 5’ to 3’ order: a first region of any defined length sequence, a self-cleaving peptide sequence, a first complementary region adjacent to a first variable region sequence, and a first variable region sequence;

(c) contacting the first nucleic acid and the second nucleic to generate a third nucleic acid;

(d) providing a fourth nucleic acid comprising in 5’ to 3’ order: a vector sequence, a second complementary region adjacent to a second variable region sequence, a second variable region sequence, a second hypervariable region sequence, the first restriction endonuclease site, and the barcode sequence;

(e) contacting the third nucleic acid and the fourth nucleic acid with a restriction endonuclease; and

(f) assembling the third nucleic acid and the fourth nucleic acid using a reaction mixture comprising one or more enzymes, comprising an exonuclease, a flap endonuclease 1, a polymerase, and a ligase.

~~15.~~9. The method of claim ~~14~~8, wherein the first restriction endonuclease site or the second restriction endonuclease site is a Type IIS restriction endonuclease (TIIS-RE) site.

~~16.~~10. The method of claim ~~14~~8, wherein the restriction endonuclease is a Type IIS restriction endonuclease.

~~17.~~11. The method of claim ~~14~~8, wherein the reaction mixture comprises a ligase.

~~18.~~12. The method of claim ~~14~~8, wherein the first hypervariable region sequence and the second hypervariable region sequence each comprises a complementary determining region (CDR), and wherein the CDR is a CDR3.

~~19. The method of claim 18, wherein the CDR is CDR3.~~

~~20.~~13. The method of claim ~~14~~8, wherein the self-cleaving peptide is P2A.

~~21.~~14. The method of claim ~~14~~8, wherein about 100 variants of the first variable region sequence are synthesized, and wherein about 130 variants of the second variable region sequence are synthesized.

~~22. The method of claim 14, wherein about 130 variants of the second variable region sequence are synthesized.~~

~~23.~~15. The method of claim ~~14~~8, further comprising amplifying the nucleic acid with a first primer complementary to a first barcode sequence and a second primer wherein at least 99% of the amplicons have no deletions.

~~24.~~16. A method for nucleic acid assembly, comprising:

(a) de novo synthesizing a first nucleic acid comprising a first variable region sequence;

(b) de novo synthesizing a second nucleic acid comprising a second variable region sequence;

(c) de novo synthesizing a third nucleic acid comprising in 5' to 3' order: a first region of fixed variability sequence, a first region of any defined length sequence, a self-cleaving peptide sequence, a first complementary region adjacent to a first variable region sequence, and a second region of fixed variability sequence; and

(d) contacting the first nucleic acid, the second nucleic acid, and the third nucleic acid with a reaction mixture comprising an exonuclease, an endonuclease, a polymerase, and a ligase,

wherein the endonuclease is a flap endonuclease 1.

~~25.~~17. The method of claim ~~24~~16, wherein the first variable region sequence or the second variable region sequence is amplified with a hypervariable region sequence.

~~26.~~18. The method of claim ~~25~~17, wherein the hypervariable region sequence comprises a CDR, and wherein the CDR is CDR3.

~~27. The method of claim 26, wherein the CDR is CDR3.~~

~~28. The method of claim 24, further comprising contacting with sequences comprising one or more regions of any defined length.~~

~~29.~~19. The method of claim ~~24~~16, wherein about 100 variants of the first variable region sequence are synthesized, and wherein about 130 variants of the second variable region sequence are synthesized.

~~30. The method of claim 24, wherein about 130 variants of the second variable region sequence are synthesized.~~

~~31.~~20. The method of claim ~~24~~16, wherein the self-cleaving peptide is P2A.

~~32. The method of claim 24, wherein the exonuclease is exonuclease III.~~

~~33. The method of claim 24, wherein the endonuclease is a flap endonuclease.~~

~~34. The method of claim 33, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.~~

~~35.~~21. The method of claim 16~~24~~, wherein the polymerase comprises 5’ to 3’ polymerase activity.

~~36. The method of claim 24, wherein the polymerase is a DNA polymerase.~~

~~37. The method of claim 24, wherein the ligase catalyzes joining of at least two nucleic acids.~~

~~38. The method of claim 24, wherein the first region of fixed variability sequence and the second region of fixed variability sequence are each about 10 to about 100 base pairs.~~

~~39. The method of claim 24, wherein the first region of fixed variability sequence and the second region of fixed variability sequence are each about 40 base pairs.~~

~~40. A method for nucleic acid assembly, comprising:~~

~~(a) providing a first nucleic acid comprising a first region of any defined length sequence;~~

~~(b) providing a second nucleic acid comprising a second region of any defined length sequence;~~

~~(c) assembling a third nucleic acid comprising in 5’ to 3’ order: a first complementary region adjacent to a first variable region sequence, a first variable region sequence, and a first hypervariable region sequence;~~

~~(d) assembling a fourth nucleic acid comprising in 5’ to 3’ order: a second complementary region adjacent to a second variable region sequence, a second variable region sequence, and a second hypervariable region sequence;~~

(e) contacting the first nucleic acid, the second nucleic acid, the third nucleic acid, and the fourth nucleic acid; and

(f) amplifying a product from step (e).

41. The method of claim 40, further comprising an error correction step.

42. The method of claim 40, further comprising contacting a reaction mixture comprising an exonuclease, an endonuclease, a polymerase, and a ligase during step (e).

43. The method of claim 40, wherein the first hypervariable region sequence and the second hypervariable region sequence each comprises a complementary determining region (CDR).

44. The method of claim 43, wherein the CDR is CDR3.

45. The method of claim 40, wherein the first nucleic acid comprises about 300 to about 700 base pairs.

46. The method of claim 40, wherein the second nucleic acid comprises about 200 to about 600 base pairs.

47. The method of claim 40, wherein the third nucleic acid comprises about 200 to about 600 base pairs.

48. The method of claim 40, wherein the fourth nucleic acid comprises about 200 to about 600 base pairs.

49. A method for nucleic acid assembly, comprising:

(a) de novo synthesizing:

i. a first nucleic acid comprising in 5' to 3' order: a first complementary region adjacent to a first variable region sequence and a first variable region sequence;

ii. a second nucleic acid comprising in 5' to 3' order: a first region of fixed variability sequence and a first hypervariable region sequence;

iii. a third nucleic acid comprising a second variable region sequence;

iv. a fourth nucleic acid comprising in 5' to 3' order: a restriction endonuclease site and a second region of fixed variability sequence; and

v. a fifth nucleic acid comprising in 5’ to 3’ order: the second region of fixed variability sequence, a second hypervariable region sequence, and a variable constant region sequence;

(b) contacting the first nucleic acid, the second nucleic acid, the third nucleic acid, the fourth nucleic acid, and the fifth nucleic acid with a reaction mixture comprising an exonuclease, an endonuclease, a polymerase, and a ligase; and

(c) cloning a construct of step (b) into a vector sequence.

50. The method of claim 49, wherein the first hypervariable region sequence and the second hypervariable region sequence each comprises a complementary determining region (CDR).

51. The method of claim 49, wherein the CDR is CDR3.

52. The method of claim 49, further comprising contacting one or more variable constant regions.

53. The method of claim 49, wherein the exonuclease is exonuclease III.

54. The method of claim 49, wherein the endonuclease is a flap endonuclease.

55. The method of claim 54, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.

56. The method of claim 49, wherein the polymerase comprises 5’ to 3’ polymerase activity.

57. A method for nucleic acid assembly, comprising:

(a) providing a first nucleic acid comprising in 5’ to 3’ order: a first complementary region adjacent to a first variable region sequence and a first variable region sequence;

(b) providing a second nucleic acid sequence comprising in 5’ to 3’ order: a first region of fixed variability sequence, a first hypervariable region sequence, a restriction endonuclease site, a second hypervariable region sequence, and a universal primer;

(c) amplifying the first nucleic acid and the second nucleic acid to generate a third nucleic acid;

(d) providing a vector sequence comprising the first complementary region adjacent to the first variable region sequence and a first region of any defined length sequence;

(e) contacting the third nucleic acid and the vector sequence;

(f) contacting a fourth nucleic acid comprising in 5' to 3' order: a self-cleaving peptide sequence, a second complementary region adjacent to a second variable region sequence, and a second variable region sequence.

58. The method of claim 57, wherein the first hypervariable region sequence and the second hypervariable region sequence each comprises a complementary determining region (CDR).

59. The method of claim 58, wherein the CDR is CDR3.

60. The method of claim 57, wherein the self-cleaving peptide is P2A.

61. A method for nucleic acid assembly, comprising:

(a) de novo synthesizing:

i. a first nucleic acid comprising a first complementary region adjacent to a first variable region sequence and a first variable region sequence;

ii. a second nucleic acid comprising a first hypervariable region sequence;

iii. a third nucleic acid comprising a second variable region sequence;

iv. a fourth nucleic acid comprising in 5' to 3' order: a first hypervariable region sequence, a first region of fixed variability, and a barcode;

(b) amplifying the first nucleic acid and the second nucleic acid to generate a fifth nucleic acid;

(c) amplifying the third nucleic acid and the fourth nucleic acid to generate a fifth nucleic acid;

(d) contacting the fifth nucleic acid and the sixth nucleic acid with a reaction mixture comprising an exonuclease, an endonuclease, a polymerase, and a ligase to generate a seventh nucleic acid;

(e) circularizing the seventh nucleic acid;

(f) sequencing and identifying the seventh nucleic acid using the barcode;

(g) amplifying the seventh nucleic acid; and

(h) assembling the seventh nucleic acid in a vector using the reaction mixture comprising the exonuclease, the endonuclease, the polymerase, and the ligase.

62. The method of claim 61, wherein the first variable region sequence or the second variable region sequence is amplified with a hypervariable region sequence.

~~63. The method of claim 62, wherein the hypervariable region sequence comprises a CDR.~~

~~64. The method of claim 63, wherein the CDR is CDR3.~~

~~65. The method of claim 61, further comprising contacting with sequences comprising one or more regions of any defined length.~~

~~66. The method of claim 61, wherein about 100 variants of the first variable region sequence are synthesized.~~

~~67. The method of claim 61, wherein about 130 variants of the second variable region sequence are synthesized.~~

~~68. The method of claim 61, wherein the self-cleaving peptide is P2A.~~

~~69. The method of claim 61, wherein the exonuclease is exonuclease III.~~

~~70. The method of claim 61, wherein the endonuclease is a flap endonuclease.~~

~~71. The method of claim 70, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.~~

~~72. The method of claim 61, wherein the polymerase comprises 5' to 3' polymerase activity.~~

~~73. The method of claim 61, wherein the polymerase is a DNA polymerase.~~

~~74. The method of claim 61, wherein the ligase catalyzes joining of at least two nucleic acids.~~

~~75. The method of claim 61, wherein the first region of fixed variability sequence and the second region of fixed variability sequence are each about 10 to about 100 base pairs.~~

~~76. The method of claim 61, wherein the first region of fixed variability sequence and the second region of fixed variability sequence are each about 40 base pairs.~~