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 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 at least 10,000 nucleic acids are assembled.
4. The method of claim 1, wherein at least 100,000 nucleic acids are assembled.
5. The method of claim 1, wherein the polymerase comprises 5’ to 3’ polymerase activity.
6. The method of claim 1, wherein the polymerase is a DNA polymerase.
7. The method of claim 1, wherein the ligase catalyzes joining of at least two nucleic acids.
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.

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

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

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

12. The method of claim 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.

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

14. The method of claim 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.

15. The method of claim 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.

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.

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

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

19. The method of claim 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.

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

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