CLAIMS

WHAT IS CLAIMED IS:

1. A method for nucleic acid assembly, comprising:
   (a) providing a plurality of polynucleotides, wherein each of the polynucleotides do not comprise a terminal region of sequence complementary to another polynucleotide of the plurality of polynucleotides; and
   (b) mixing the plurality of polynucleotides with a 3' exonuclease, a flap endonuclease, a polymerase, and a ligase, wherein the plurality of polynucleotides are annealed in a processive predetermined order based on a complementary sequence between adjacent polynucleotides.

2. The method of claim 1, wherein the 3' exonuclease is exonuclease III.

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

4. The method of claim 1, wherein the polymerase comprises 5' to 3' polymerase activity.

5. The method of claim 1, wherein the polymerase is a DNA polymerase.

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

7. The method of claim 4, wherein a concentration of the flap endonuclease 1 is in a range of about 0.32U to about 4.8U.

8. The method of claim 2, wherein a concentration of the exonuclease III is in a range of about 0.1U to about 10U.

9. The method of claim 5, wherein a concentration of the polymerase is in a range of about 0.01U to about 2U.

10. The method of claim 7, wherein a concentration of the ligase is up to about 2.0U.

11. The method of claim 7, wherein a concentration of the ligase is in a range of about 4.0U to about 8.0U.

12. A method for nucleic acid assembly, comprising:
   (a) providing a first double stranded nucleic acid;

(b) providing a second double stranded nucleic acid;

(c) providing a third double stranded nucleic acid comprising in 5' to 3' order: a 5' flanking adapter sequence, a first complementary sequence to the first double stranded nucleic acid, a second complementary sequence to the second double stranded nucleic acid, and a 3' flanking adapter sequence, wherein the first double stranded nucleic acid, the second double stranded nucleic acid, and the third double stranded nucleic acid comprise non-homologous sequences at terminal regions; and

(d) mixing the first double stranded nucleic acid, the second double stranded nucleic acid, and the third double stranded nucleic acid with a reaction mixture comprising a 3' exonuclease, a flap endonuclease, a polymerase, and a ligase.

13. A method for nucleic acid assembly, comprising:

(a) providing a first double stranded nucleic acid comprising in 5' to 3' order: a 5' flanking adapter sequence, a complementary sequence, an insert sequence, and a 3' flanking adapter sequence;

(b) providing a second double stranded nucleic acid comprising in 5' to 3' order: a 5' flanking adapter sequence, a complementary sequence, an insert sequence, and a 3' flanking adapter sequence; and

(c) mixing the first double stranded nucleic acid and the second double stranded nucleic acid with a reaction mixture comprising a 3' exonuclease, a flap endonuclease, a polymerase, and a ligase.

14. The method of claim 1, wherein step (b) occurs at a temperature of about 30 ºC to about 60 ºC.

15. The method of claim 1, wherein mixing the plurality of polynucleotides with the flap endonuclease results in a 5' overhang.

16. The method of claim 1, wherein the flap endonuclease is flap endonuclease 1.

17. The method of claim 1, wherein the 3' exonuclease is exonuclease III; the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1; and the polymerase is a DNA polymerase.

18. The method of claim 1, wherein a concentration of the 3' exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is in a range of about 4.0U to about 8.0U.

19. The method of claim 1, wherein a concentration of the 3’ exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is up to about 2.0U.

20. The method of claim 12, wherein the 3’ exonuclease is exonuclease III.

21. The method of claim 12, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.

22. The method of claim 12, wherein the polymerase is a DNA polymerase.

23. The method of claim 12, wherein a concentration of the 3’ exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is in a range of about 4.0U to about 8.0U.

24. The method of claim 12, wherein a concentration of the 3’ exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is up to about 2.0U.

25. The method of claim 13, wherein the 3’ exonuclease is exonuclease III.

26. The method of claim 13, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1.

27. The method of claim 13, wherein the polymerase is a DNA polymerase.

28. The method of claim 13, wherein a concentration of the 3’ exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is in a range of about 4.0U to about 8.0U.

29. The method of claim 13, wherein a concentration of the 3’ exonuclease is in a range of about 0.1U to about 10U; a concentration of the flap endonuclease is in a range of about 0.32U to about 4.8U; a concentration of the polymerase is in a range of about 0.01U to about 2U; and a concentration of the ligase is up to about 2.0U.