CLAIMS

WHAT WE CLAIM IS:

1. A method for nucleic acid assembly comprising:

providing at least 10 different nucleic acid fragments, wherein each of the at least 10 different nucleic acid fragments does not comprise a terminal region of sequence homology to another fragment of the at least 10 different nucleic acid fragments; and

mixing the at least 10 different nucleic acid fragments with a plurality of enzymes, wherein the plurality of enzymes is selected from an endonuclease, exonuclease, a polymerase, and a ligase to form a nucleic acid; and wherein the endonuclease is a flap endonuclease.

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

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

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

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

6. The method of claim 4, wherein a concentration of the flap endonuclease 1 provided is less than about 5.0 U.

7. The method of claim 1, wherein the polymerase comprises 5′ to 3′ polymerase activity.

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

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

10. The method of claim 1, wherein a concentration of the polymerase is in a range of about 0.01 U to about 2 U.

11. The method of claim 1, wherein a concentration of the ligase is up to about 2.0 U.

12. The method of claim 1, wherein a concentration of the ligase is in a range of about 4.0 U to about 8.0 U.

13. The method of claim 1, wherein mixing occurs at a temperature of about 30°C. to about 60°C.

14. The method of claim 1, wherein mixing the at least 10 different fragments with the endonuclease results in a 5′ overhang.

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

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

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

18. A method for nucleic acid assembly, comprising:

providing a plurality of polynucleotides, wherein each of the polynucleotides do not comprise a terminal region of sequence homology to another polynucleotide of the plurality of polynucleotides; and

mixing the plurality of polynucleotides with a 3′ to 5′ exonuclease, a thermostable endonuclease, a high fidelity polymerase, and a thermostable ligase, wherein the plurality of

polynucleotides are annealed in a processive predetermined order based on a complementary sequence between adjacent polynucleotides.

19. The method of claim 18, wherein the 3′ to 5′ exonuclease is exonuclease III.

20. The method of claim 19, wherein a concentration of the exonuclease III is in a range of about 0.1 U to about 10 U.

21. The method of claim 18, wherein the thermostable endonuclease is a thermostable flap endonuclease.

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

23. The method of claim 22, wherein a concentration of the flap endonuclease 1 is in a range of about 0.32 U to about 4.8 U.

24. The method of claim 22, wherein a concentration of the flap endonuclease 1 provided is less than about 5.0 U.

25. The method of claim 18, wherein the high fidelity polymerase comprises 5′ to 3′ polymerase activity.

26. The method of claim 18, wherein the high fidelity polymerase is a DNA polymerase.

27. The method of claim 18, wherein the thermostable ligase catalyzes joining of at least two nucleic acids.

28. The method of claim 18, wherein a concentration of the high fidelity polymerase is in a range of about 0.01 U to about 2 U.

29. The method of claim 18, wherein a concentration of the thermostable ligase is up to about 2.0 U.

30. The method of claim 18, wherein a concentration of the thermostable ligase is in a range of about 4.0 U to about 8.0 U.

31. The method of claim 18, wherein mixing occurs at a temperature of about 30° C. to about 60° C.

32. The method of claim 18, wherein mixing the plurality of polynucleotides with the thermostable endonuclease results in a 5′ overhang.

33. The method of claim 18, wherein the 3′ to 5′ exonuclease is exonuclease III; the thermostable endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1; and the high fidelity polymerase is a DNA polymerase.

34. The method of claim 18, wherein a concentration of the 3′ to 5′ exonuclease is in a range of about 0.1 U to about 10 U; a concentration of the thermostable endonuclease is in a range of about 0.32 U to about 4.8 U; a concentration of the high fidelity polymerase is in a range of about 0.01 U to about 2 U; and a concentration of the thermostable ligase is in a range of about 4.0 U to about 8.0 U.

35. The method of claim 18, wherein a concentration of the 3′ to 5′ exonuclease is in a range of about 0.1 U to about 10 U; a concentration of the thermostable endonuclease is in a range of about 0.32 U to about 4.8 U; a concentration of the high fidelity polymerase is in a range of about 0.01 U to about 2 U; and a concentration of the thermostable ligase is up to about 2.0 U.

36. The method of claim 18, wherein the ratio of the thermostable endonuclease to the 3′ to 5′ exonuclease is from about 0.1:1 to about 1:5.

37. The method of claim 18, wherein the ratio of the thermostable endonuclease to the 3′ to 5′ exonuclease is at least 1:1.