METHODS FOR SEAMLESS NUCLEIC ACID ASSEMBLY

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 62/663,089 filed April 26, 2018; and U.S. Provisional Patent Application No. 62/518,496 filed on June 12, 2017, each of which is incorporated herein by reference in its entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 6, 2018, is named 44854-746_601_SL.txt and is 1,585 bytes in size.

BACKGROUND

[0003] De novo nucleic acid synthesis is a powerful tool for basic biological research and biotechnology applications. While various methods are known for the synthesis of relatively short fragments of nucleic acids on a small scale, these techniques suffer from scalability, automation, speed, accuracy, and cost. Thus, a need remains for efficient methods of seamless nucleic acid assembly.

BRIEF SUMMARY

[0003a] Provided herein are method for nucleic acid assembly, comprising: (a) providing a plurality of double-stranded polynucleotides, comprising in 5' to 3' order: a 5' flanking adapter sequence, a complementary sequence, an insert sequence, and a 3' flanking adapter sequence, wherein each of the double-stranded polynucleotides do not comprise a terminal region complementary to another polynucleotide of the plurality of double-stranded polynucleotides; and (b) mixing the plurality of double-stranded polynucleotides with a 3' exonuclease, a flap endonuclease, a polymerase, and a ligase, wherein the plurality of double-stranded polynucleotides are annealed in a processive predetermined order based on the complementary sequence between adjacent polynucleotides.

[0003b] Provided herein are methods 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-complementary 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.

[0003c] Provided herein are methods 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.

[0004] Provided herein are methods for nucleic acid synthesis and assembly, comprising: (a) providing a plurality of polynucleotides; and (b) mixing the plurality of polynucleotides with an 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. Further provided herein are methods for nucleic acid synthesis and assembly, wherein the exonuclease is exonuclease III. Further provided herein are methods for nucleic acid synthesis and assembly, wherein the flap endonuclease is flap endonuclease 1, exonuclease 1, XPG, Dna2, or GEN1. Further provided herein are methods for nucleic acid synthesis and assembly, wherein the polymerase comprises 5' to 3' polymerase activity. Further provided herein are methods for nucleic acid synthesis and assembly, wherein the polymerase is a DNA polymerase. Further provided herein are methods for nucleic acid synthesis and assembly, wherein the ligase catalyzes joining of at least two nucleic acids. Further provided herein are methods for nucleic acid synthesis and assembly, wherein a concentration of the flap endonuclease 1 is in a range of about 0.32U to about 4.8U. Further provided herein are methods for nucleic acid synthesis and assembly, wherein a concentration of the exonuclease III is in a range

CLAIMS

WHAT IS CLAIMED IS:

1. A method for nucleic acid assembly, comprising:
   (a) providing a plurality of double-stranded polynucleotides, comprising in 5’ to 3’ order: a 5’ flanking adapter sequence, a complementary sequence, an insert sequence, and a 3’ flanking adapter sequence, wherein each of the double-stranded polynucleotides do not comprise a terminal region complementary to another polynucleotide of the plurality of double-stranded polynucleotides; and
   (b) mixing the plurality of double-stranded polynucleotides with a 3’ exonuclease, a flap endonuclease, a polymerase, and a ligase, wherein the plurality of double-stranded polynucleotides are annealed in a processive predetermined order based on the 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 the flap endonuclease is flap endonuclease 1 and a concentration of the flap endonuclease 1 is in a range of 0.32U to 4.8U.

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

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

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

11. The method of claim 7, wherein a concentration of the ligase is in a range of 4.0U to 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-complementary 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 30 ºC to 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 0.1U to 10U; a concentration of the flap endonuclease is in a range of 0.32U to 4.8U; a concentration of the polymerase is in a range of 0.01U to 2U; and a concentration of the ligase is up to 2.0U.

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

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

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