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] In one aspect, provided herein is 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.

[0003b] In one aspect, provided herein is 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.

[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