CLAIMS

WHAT IS CLAIMED IS:

1. A method of assembling a DNA molecule having a desired sequence comprising:

a) contacting a DNA ligase with a plurality of short oligonucleotides comprising at least a portion of the desired sequence of the DNA molecule to be assembled to create a mixture,

wherein at least a portion of the short oligonucleotides in the plurality overlap with and are complementary to a portion of the sequence of at least one other short oligonucleotide in the plurality, and wherein at least two of the short oligos abut one another when bound to their complementary sequence(s), and at least 50% of the short oligonucleotides in the plurality are 6-30 nucleotides in length;

b) performing a ligase chain reaction on the mixture to thereby generate a set of polynucleotides, and

c) contacting the set of polynucleotides with a DNA polymerase and dNTPs to join the set of polynucleotides, and to thereby assemble the DNA molecule having the desired sequence.

2. The method of claim 1 wherein the set of polynucleotides is joined by a DNA assembly reaction.

3. The method of claim 1 wherein the set of polynucleotides is joined by polymerase cycling assembly.

4. The method of any one of claims 1-3 further comprising contacting the plurality of short oligonucleotides with a kinase before or simultaneous with the contacting of the short oligonucleotides with the DNA ligase.

5. The method of any one of claims 1-4 wherein the DNA ligase is a thermostable DNA ligase; or is a Taq ligase.

6. The method of any one of claims 1-5 wherein the abutting short oligos comprise a phosphorylated 5’ terminal nucleotide of a first short oligo and a 3’ hydroxyl on a 3’ terminal nucleotide of a second short oligo.

7. The method of any one of claims 1-6 further comprising amplifying the DNA molecule having the desired sequence using the polymerase chain reaction.

8. The method of any one of claims 1-7 wherein the method is performed in solution.

9. The method of any one of claims 1-8 wherein the DNA molecule having the desired sequence is 100-10,000 base pairs in length; or the DNA molecule having the desired sequence is 100-5,000 base pairs in length.

10. The method of any one of claims 1-9 wherein the desired sequence further comprises a universal 5′ flanking sequence and a universal 3′ flanking sequence; or further comprises combining multiple pools of polynucleotides to join the polynucleotides via polymerase cycling assembly.

11. The method of any one of claims 1-10 further comprising at least 10 cycles of the polymerase chain reaction to amplify the DNA molecule having the desired sequence; or

wherein the plurality of short oligonucleotides comprises at least 10 short oligonucleotides; or

wherein the plurality of short oligonucleotides comprises at least 20 short oligonucleotides; or

wherein the plurality of short oligonucleotides comprises from 2 to 250 short oligonucleotides; or

wherein the plurality of short oligonucleotides are 6-18 nucleotides in length; or

wherein at least 50% of the short oligonucleotides in the plurality are 6-18 nucleotides in length.

12. The method of claim 11 wherein at least 75% of the oligonucleotides in the plurality are 6-18 nucleotides in length at the start of the method.

13. The method of any one of claims 1-12, wherein the method is performed without the use of restriction enzymes.

14. The method of any one of claims 1-13 wherein assembled DNA molecule of desired sequence has an error rate of fewer than 1 error per 2,000 base pairs; or fewer than 1 error per 14,000 base pairs.

15. The method of any one of claims 1-14 wherein the error rate is obtained in the method without the use of error correction enzymes.

16. The method of any one of claims 1-15 wherein the plurality of short oligonucleotides comprises at least 15 pools of oligonucleotides.

17. The method of any one of claims 1-16 wherein the assembly is done entirely in vitro.

18. The method of any one of claims 1-17 wherein the oligonucleotides are 8-mers or 16-mers.

19. The method of any one of claims 1-18 wherein the DNA molecule is assembled with scarless assembly.

20. The method of any one of claims 1-19 wherein the plurality of short oligonucleotides comprises more than 64 short oligonucleotides.

21. The method of any one of claims 1-20 wherein the method is conducted without the use of linker, adaptor, or spacer DNA molecules ; or

wherein the ligase chain reaction comprises at least 5 cycles of denaturation, annealing, and ligation ; or

wherein the plurality of short oligonucleotides comprises more than 64 short oligonucleotides ; or

wherein less than 10% of the oligonucleotides in the mixture are longer than 20 nucleotides in length ; or

wherein less than 1% of the oligonucleotides in the mixture are longer than 20 nucleotides in length.