What is claimed is:

1. A method of producing synthetic circular supercoiled DNA (^{SCS}DNA), the method comprising:

introducing at least two precursor DNA fragments into a buffer medium comprising a thermostable DNA ligase enzyme, wherein the precursor DNA fragments will correctly assemble to generate a defined DNA sequence;

applying heat to a first temperature to cause the at least two precursor DNA fragments to denature; and

lowering the temperature to a second temperature for: (i) annealing in the presence of the thermostable DNA ligase enzyme, thereby generating double-stranded DNA heteroduplexes formed by base pairing of complementary regions, a portion of the heteroduplexes having single-stranded 5' overhangs and a portion of the heteroduplexes having 3' overhangs, wherein when the 5' intramolecular overhangs on a heteroduplex molecule are complementary and ligation on both DNA strands occurs, ^{SCS}DNA is produced, and when the 3' intramolecular overhangs on a heteroduplex molecule are complementary and ligation on both DNA strands occurs, ^{SCS}DNA is produced; and (ii) substantial supercoiling of the ^{SCS}DNA in the presence of the thermostable type II topoisomerase.

2. The method of claim 1, wherein the method of producing ^{SCS}DNA is a one-pot method.

3. The method of claims 1 or 2, wherein the at least two precursor DNA fragments are selected from double stranded (ds) DNA molecules, single-stranded DNA molecules, DNA oligonucleotides, or mixtures thereof.

4. The method of any of the preceding claims, wherein the first temperature is in a range from about 37°C to about 100°C, preferably about 80°C to about 100°C, and wherein the first temperature is applied for time in a range from about 30 seconds to about 10 minutes, preferably about 1 minute to about 5 minutes.

5. The method of any of the preceding claims, wherein the second temperature is about 25°C to about 85°C, preferably 50°C to about 70°C, and wherein the second temperature is maintained for time in a range from about 0.1 minutes to about 60 minutes.

6. The method of any of the preceding claims, wherein the application of heat to a first temperature to denature and the lowering to a second temperature to anneal is repeatedly performed in cycles, wherein the number of cycles is in a range from 2 cycles to 100 cycles.

7. The method of any of the preceding claims, wherein during annealing, heteroduplex double-stranded DNA sequences are generated, formed by base pairing of complementary regions, wherein the heteroduplexes comprise 5' or 3' overhangs.

8. The method of claim 7, wherein ^{SCS}DNA is produced when the 5' or 3' overhangs are complementary.

9. The method of any of the preceding claims, wherein the buffer comprises a combination of ATP, Tris-HCl, $MgCl_2$, KCl, NaCl, DTT, beta-mercaptoethanol, NAD, and TRITON X-100, and wherein the pH is maintained at about 4 to about 12, preferably about 7.5 to about 9.

10. The method of claim 3, wherein two precursor DNA fragments are used and wherein a portion of the ^{SCS}DNA undergoes negative supercoiling in the presence of the thermostable DNA ligase enzyme.

11. The method of any of the preceding claims, wherein the buffer medium further comprises a type II topoisomerase, preferably a thermostable type II topoisomerase, and wherein the ^{SCS}DNA are substantially supercoiled.

12. The method of claim 11, wherein the type II topoisomerase is thermostable and the thermostable type II isomerase can be cycled through the denaturing and annealing processes while maintaining some or all of its activity, wherein the type II topoisomerase is a DNA gyrase or a Topoisomerase IV.

13. The method of claim 11, wherein the type II topoisomerase is thermostable or not thermostable and the method further comprises lowering the temperature to a third temperature and adding a bolus of type II topoisomerase to initiate substantial supercoiling of the ^{SCS}DNA in the presence

of the type II topoisomerase, wherein the type II topoisomerase is a DNA gyrase or a Topoisomerase IV.

14. The method of any of the preceding claims, wherein the at least two precursor DNA fragments do not comprise intentional nicks, no bending proteins are added, the ^{SCS}DNA produced is devoid of bacterial DNA, a single-stranded DNA binding protein is added, a thermostable single-stranded DNA binding protein is added, or any combination thereof.

15. A synthetic circular supercoiled DNA (^{SCS}DNA) produced according to the method of any of claims 1-14.