What is claimed is:

1. A method for ligation-coupled polymerase chain reaction (PCR), comprising:

(i) providing a partially double-stranded DNA substrate comprising a first strand and a second strand, the partially double-stranded DNA substrate comprising a first 3' overhang, a double-stranded portion, and a second 3' overhang,

the first strand comprising, in a 5' to 3' direction: a first 5' end, a first portion, and a second portion,

the second strand comprising, in a 5' to 3' direction: a second 5' end, a third portion, and a fourth portion of the partially double-stranded DNA substrate,

wherein the first portion of the first strand and the third portion of the second strand are complementary and form the double-stranded portion,

wherein the second portion of the first strand forms the first 3' overhang,

wherein the fourth portion of the second strand forms the second 3' overhang,

wherein the second portion of the first strand and the fourth portion of the second strand each comprise a first common nucleotide sequence positioned at a 5' end of the first 3' overhang and the second 3' overhang, respectively, and

wherein the second portion of the first strand and the fourth portion of the second strand each comprise a second common nucleotide sequence positioned 3' to the first common nucleotide sequence;

(ii) adding a plurality of first indexing primers, a plurality of second indexing primers, a ligase, a DNA polymerase and deoxynucleotide triphosphates (dNTPs) to the partially double-stranded DNA substrate to yield a first reaction mixture, wherein each of the plurality of first indexing primers comprise a first 3' terminal portion complementary to the first common nucleotide sequence, and wherein each of the plurality of second indexing primers comprise a second 3' terminal portion complementary to the first common nucleotide sequence and a first 5' portion positioned 5' to the second 3' terminal portion and complementary to the second common nucleotide sequence;

(iii) incubating the first reaction mixture under a first set of conditions comprising a ligation temperature for a ligation duration, wherein the first set of conditions is sufficient:

a) for the first 3' terminal portion to anneal to the first common nucleotide sequence, and

b) for the ligase to ligate one of the plurality of first indexing primers to the first 5’ end of the first strand and one of the plurality of first indexing primers to the second 5’ end of the second strand to yield a second reaction mixture comprising:

a third strand comprising, in a 5’ to 3’ direction, one of the plurality of first indexing primers, the first portion, and the second portion, and

a fourth strand comprising, in a 5’ to 3’ direction, one of the plurality of first indexing primers, the third portion, and the fourth portion;

(iv) incubating the second reaction mixture under a second set of conditions comprising a first denaturation temperature for a first denaturation duration, a first annealing temperature for a first annealing duration, and a first extension temperature for a first extension duration, wherein the second set of conditions is sufficient:

a) to inactivate the ligase, denature double-stranded DNA, and, optionally, to activate the DNA polymerase,

b) for the second 3’ terminal portion and the first 5’ portion of one of the plurality of second indexing primers to anneal to at least the first common nucleotide sequence and the second common nucleotide sequence of the second portion of the third strand and for the second 3’ terminal portion and the first 5’ portion of one of the plurality of second indexing primers to anneal to at least the first common nucleotide sequence and the second common nucleotide sequence of the fourth portion of the fourth strand, and

c) for the DNA polymerase to extend the one of the plurality of second indexing primers annealed to the first common nucleotide sequence and second common nucleotide sequence of the second portion of the third strand and for the DNA polymerase to extend the one of the plurality of second indexing primers annealed to the first common nucleotide sequence and second common nucleotide sequence of the fourth portion of the fourth strand, to yield a third reaction mixture comprising the third strand, the fourth strand, a fifth strand, and a sixth strand, the fifth strand comprising, in a 5’ to 3’ direction, one of the plurality of second indexing primers, the third portion, and a reverse complement of one of the plurality of first indexing primers, the sixth strand comprising, in a 5’ to 3’ direction, one of the plurality of second indexing primers, the first portion, and the reverse complement of one of the plurality of first indexing primers;

(v) incubating the third reaction mixture under a third set of conditions comprising a second denaturation temperature for a second denaturation duration, a second annealing temperature for a second annealing duration, and a second extension temperature for a second extension duration, wherein the third set of conditions is sufficient

a) to denature double-stranded DNA,

b) for one of the plurality of first indexing primers to anneal to the reverse complement of one of the plurality of first indexing primers of the fifth strand and for one of the plurality of first indexing primers to anneal to the reverse complement of one of the plurality of first indexing primers of the sixth strand, and

c) for the DNA polymerase to extend the one of the plurality of first indexing primers annealed to the reverse complement of one of the plurality of first indexing primers of the fifth strand and the one of the plurality of first indexing primers annealed to the reverse complement of the one of the plurality of first indexing primers of the sixth strand, to yield a fourth reaction mixture comprising the fifth strand, the sixth strand, a seventh strand, and an eighth strand, the seventh strand comprising, in a 5' to 3' direction, one of the plurality of first indexing primers, the first portion, and a reverse complement of one of the plurality of second indexing primers, the eighth strand comprising, in a 5' to 3' direction, one of the plurality of first indexing primers, the third portion, and the reverse complement of one of the plurality of second indexing primers, wherein the seventh strand is complementary to the fifth strand, and wherein the eighth strand is complementary to the sixth strand; and

(vi) incubating the fourth reaction mixture under a fourth set of conditions comprising a third denaturation temperature for a third denaturation duration, a third annealing temperature for a third annealing duration, and a third extension temperature for a third extension duration, wherein the fourth set of conditions is sufficient for at least a portion of the plurality of first indexing primers and at least a portion of the plurality of second indexing primers to amplify the fifth strand and seventh strand and the sixth strand and eighth strand.

2. The method of claim 1, wherein steps (i) through (vi) are performed in a single closed tube.

3. The method of claim 1, wherein the partially double-stranded DNA substrate has a length of about 24 bases to about 6000 bases, wherein the first portion and the third portion of the first strand and the second strand, respectively, each have a length of about 20 bases to about 6000 bases, wherein the second portion of the first strand and the fourth portion of the second strand

each have a length of about 4 bases to about 100 bases, wherein the first common nucleotide sequence has a length of about 1 base to about 50 bases, wherein the second common nucleotide sequence has a length from about 5 bases to about 30 bases, and wherein each of the plurality of first indexing primers has a length from about 20 bases to about 100 bases, and wherein each of the plurality of second indexing primers has a length from about 20 bases to about 100 bases.

4. The method of claim 1, wherein the first common nucleotide sequence and the first 3' terminal portion have a melting temperature (T_m) greater than the ligation temperature, wherein the ligation temperature is less than a melting temperature (T_m) of the partially double-stranded DNA substrate, wherein a melting temperature (T_m) of the third strand and fourth strand is less than the first denaturing temperature, and wherein the first common nucleotide sequence, the first 3' terminal portion, and the second 3' terminal portion have a T_m less than the first annealing temperature.

5. The method of claim 1, wherein the ligation temperature is from about 25 ^oC to about 40 ^oC.

6. The method of claim 1, wherein the ligation duration is from about 5 minutes to about 60 minutes.

7. The method of claim 1, wherein the ligase is a thermolabile ligase capable of ligation in a low magnesium buffer.

8. The method of claim 1, wherein the ligase is T3 DNA ligase.

9. The method of claim 8, wherein the ligase is added at about 30 to about 300 enzyme units per 50 µL of the first reaction mixture.

10. The method of claim 1, wherein the ligase is temperature-sensitive, and wherein the first denaturation temperature for the first denaturation duration in step (iv)(a) is sufficient to inactivate the ligase.

11. The method of claim 1, wherein the DNA polymerase is a thermostable DNA polymerase with 3'-5' exonuclease proofreading activity selected from the group consisting of Kapa HiFi DNA Polymerase (Roche), NEB Q5 DNA Polymerase (NEB), PrimeStar GXL DNA Polymerase (Takara) and High Fidelity DNA Polymerase (Qiagen).

12. The method of claim 1, wherein the DNA polymerase is not active at the ligation temperature.

13. The method of claim 12, wherein the DNA polymerase further comprises a hot start antibody or aptamer, and wherein the hot start antibody or aptamer increases an activation temperature of the DNA polymerase.

14. The method of claim 13, wherein the DNA polymerase is selected from the group consisting of Kapa HiFi Hot Start DNA Polymerase (Roche), NEB Q5 Hot Start DNA Polymerase (NEB), PrimeStar GXL Hot Start DNA Polymerase (Takara) and High Fidelity Hot Start DNA Polymerase (Qiagen).

15. The method of claim 1, wherein the DNA polymerase is a hot start polymerase, and wherein the first denaturation temperature for the first denaturation duration in step (iv)(a) is sufficient to activate the hot start polymerase.

16. The method of claim 1, wherein a melting temperature (T_m) of each of the plurality of second indexing primers and the second portion or the fourth portion of the first strand and second strand, respectively, is higher than a T_m of each of the plurality of first indexing primers and the second portion or the fourth portion of the first strand and the second strand.

17. The method of claim 1, wherein the first denaturation temperature, the second denaturation temperature, and the third denaturation temperature are each independently about 95 ^oC to about 98 ^oC, and wherein the first denaturation duration, the second denaturation duration, and the third denaturation duration are each independently from about 30 seconds to about 2 minutes.

18. The method of claim 1, wherein the first annealing temperature, the second annealing temperature, and the third annealing temperature are each independently about 55 ^oC to about 65 ^oC, and wherein the first annealing duration, the second annealing duration, and the third annealing duration are each independently about 10 seconds to about 60 seconds.

19. The method of claim 1, wherein the first extension temperature, the second extension temperature, and the third extension temperature are each independently about 62 ^oC to about 72 ^oC, and wherein the first extension duration, the second extension duration, and the third extension duration are each independently about 30 seconds to about 5 minutes.

20. The method of claim 1, wherein the plurality of first indexing primers and the plurality of second indexing primers are each independently added to the first reaction mixture at about 100 nM to about 1 µM.