

WHAT IS CLAIMED:

1. A method of generating an asymmetric closed-ended double stranded nucleic acid template from a double stranded nucleic acid template having free 5' and 3' ends, comprising:
 - (A) attaching a first nucleic acid-based hairpin or dumbbell adaptor to the 3' ends of a double stranded nucleic acid template comprising free 5' and 3' ends;
 - (B) extending from each 3' end of the nucleic acid hairpin or dumbbell sequence complementary to the double stranded nucleic acid template using a processive polymerase to generate two long hairpin duplex templates, wherein one end of the duplex template comprises a closed hairpin (the "hairpin end") and the other end of the duplex comprises a free 3'-strand end and a free 5'-strand end (the "free end"); and
 - (C) closing the free end of each long hairpin duplex template to form an asymmetric closed-ended double stranded nucleic acid template by:
 - (i) ligating a second nucleic acid-based hairpin or dumbbell adaptor to the free end of the duplex template; or
 - (ii) using a TelN Protelomerase to close the free end of the duplex template, wherein the free end of the duplex template is designed to include a TelN recognition sequence.
2. The method of claim 1, wherein the double stranded nucleic acid template is a double stranded DNA template.
3. The method of claim 1 or claim 2, wherein the 5' and 3' ends of the double stranded nucleic acid template are dephosphorylated and end repaired.
4. The method of any one of the preceding claims, wherein the double stranded nucleic acid template has blunt 5' and 3' ends.
5. The method of any one of claims 1 to 3, wherein the double stranded nucleic acid template has A-tailed 3' ends.

6. The method of any one of the preceding claims, wherein the first nucleic acid-based hairpin or dumbbell adaptor is ligated to the 3' ends of the double stranded nucleic acid template using a ligase.
7. The method of claim 6, wherein the ligase is a T4 DNA Ligase or a T3 DNA ligase.
8. The method of any one of the preceding claims, wherein the first nucleic acid-based hairpin or dumbbell adaptor comprises blunt ends or T-tailed ends.
9. The method of any one of claims 1 to 7, wherein the first nucleic acid-based hairpin adaptor is a Y-shaped adaptor.
10. The method of any one of the preceding claims, wherein dimers formed from two first nucleic acid-based hairpin or dumbbell adaptors being bound to each other are removed by using size-selection or size-exclusion techniques.
11. The method of any one of the preceding claims, wherein the processive polymerase is Phi29 polymerase.
12. The method of any one of the preceding claims, wherein the second nucleic acid-based hairpin or dumbbell adaptor comprises blunt ends or T-tailed ends.
13. The method of any one of claims 1 to 11, wherein prior to step (C) the long hairpin duplex templates are digested with a restriction enzyme that creates a 5' overhang.
14. The method of any one of claims 1 to 11, wherein prior to step (C) the long hairpin duplex templates are digested with a restriction enzyme that creates a 3' overhang.
15. The method of claims 13 or 14, wherein the second nucleic acid-based hairpin or dumbbell adaptor comprises an overhang

sequence that is complementary to the overhang sequence of the digested long hairpin duplex templates.

16. The method of any one of the preceding claims, wherein the second nucleic acid-based hairpin or dumbbell adaptor are ligated to the free end of the duplex template using a polynucleotide kinase and a ligase.

17. The method of any one of the preceding claims, wherein dimers formed from two second nucleic acid-based hairpin or dumbbell adaptors being bound to each other are removed by using size-selection or size-exclusion techniques.

18. The method of any one of claims 1 to 11, wherein the TelN Protelomerase is from phage N15, and the TelN Protelomerase cuts the long hairpin duplex templates at the TelN recognition sequence and leaves covalently closed ends at the site of cleavage.

19. The method of any one of the preceding claims, wherein the method further comprises the step of:

(C') generating nanoball complexes comprising polycistronic amplified asymmetric closed-ended double stranded nucleic acid templates using rolling circle replication.

20. The method of any one of the preceding claims, wherein the method further comprises the step of:

(D) sequencing the asymmetric closed-ended double stranded nucleic acid template or nanoball complexes using a sequencing primer and a polymerase.

21. The method of claim 20, wherein step (B) step (C) (ii) and step (D) can be combined together as a one-pot reaction.

22. The method of claim 20, wherein step (B), step (C) (ii) and step (D) are carried out in a well of an automated sequencing platform.

23. A method of generating an asymmetric double stranded nucleic acid template from tagmented DNA comprising complementary hairpin loops, comprising:

(I) generating tagmented DNA comprising complementary hairpin loops at the 5' ends of each strand, wherein the hairpin loops comprise a base paired transposase recognition sequence, and wherein there is a gap of single stranded sequence between the 5' ends and the 3' ends of the tagmented DNA;

(II) filling in the gaps between the 5' ends and the 3' ends of the tagmented DNA using a gap-fill-ligation reaction to form closed ended tagmented DNA;

(III) generating a nick in the top strand at each hairpin region of the closed ended tagmented DNA;

(IV) extending from each nick a sequence complementary to the double stranded nucleic acid template using a processive polymerase to generate two long hairpin duplex templates, wherein one end of the duplex template comprises a closed hairpin (the "hairpin end") and the other end of the duplex comprises a 3'-strand end and a 5'-strand end (the "free end"); and

(V) closing the free end of each long hairpin duplex template to form an asymmetric closed-ended double stranded nucleic acid template by:

(a) ligating a nucleic acid-based hairpin or dumbbell adaptor to the free end of the duplex template; or

(b) using a TelN Protelomerase to close the free end of the duplex template, wherein the free end of the duplex template is designed to include a TelN recognition sequence.

24. The method of claim 23, wherein the transposase recognition sequence is a 19-bp Mosaic End sequence.

25. The method of claim 24, wherein the gap is 9 base pairs in length.

26. The method of any of claims 23 to 25, wherein the gap-fill-ligation reaction comprises a Klenow fragment.

27. The method of any of claims 23 to 25, wherein the gap-fill-ligation reaction comprises T4 DNA polymerase and Ampligase.
28. The method of any of claims 23 to 27, wherein the nick is generated by using a site-specific endonuclease.
29. The method of any of claims 23 to 28, wherein the processive polymerase is Phi29 polymerase.
30. The method of any of claims 23 to 29, wherein the nucleic acid-based hairpin or dumbbell adaptor comprises blunt ends or T-tailed ends.
31. The method of any one of claims 23 to 29, wherein prior to step (V) the long hairpin duplex templates are digested with a restriction enzyme that creates a 5' overhang.
32. The method of any one of claims 23 to 29, wherein prior to step (V) the long hairpin duplex templates are digested with a restriction enzyme that creates a 3' overhang.
33. The method of claims 31 or 32, wherein the nucleic acid-based hairpin or dumbbell adaptor comprises an overhang sequence that is complementary to the overhang sequence of the digested long hairpin duplex templates.
34. The method of any one of claims 23 to 33, wherein the nucleic acid-based hairpin or dumbbell adaptor are ligated to the free end of the duplex template using a polynucleotide kinase and a ligase.
35. The method of any one of the preceding claims, wherein dimers formed from two nucleic acid-based hairpin or dumbbell adaptors being bound to each other are removed by using size-selection or size-exclusion techniques.
36. The method of any one of claims 23 to 29, wherein the TelN Protelomerase is from phage N15, and the TelN Protelomerase cuts the

long hairpin duplex templates at the TelN recognition sequence and leaves covalently closed ends at the site of cleavage.

37. The method of any one of claims 23 to 36, wherein the method further comprises the step of:

(V') generating nanoball complexes comprising polycistronic amplified asymmetric closed-ended double stranded nucleic acid templates using rolling circle replication.

38. The method of any one of claims 23 to 37, wherein the method further comprises the step of:

(VI) sequencing the asymmetric closed-ended double stranded nucleic acid template or nanoball complexes using a sequencing primer and a polymerase.

39. The method of claim 38, wherein step (V)(ii) and step (VI) can be combined together into a single step.

40. The method of claim 39, wherein step (V)(ii) and step (VII) are carried out in a well of an automated sequencing platform.

41. A method of sequencing tagmented DNA comprising complementary hairpin loops, comprising:

(I) generating tagmented DNA comprising complementary hairpin loops at the 5' ends of each strand, wherein the hairpin loops comprise a base paired transposase recognition sequence, and wherein there is a gap of single stranded sequence between the 5' ends and the 3' ends of the tagmented DNA;

(II) forming two extended strands of tagmented DNA comprising a transposase recognition sequence and complementary transposase recognition sequence at 5' and 3' ends of each extended strand of tagmented DNA by using a polymerase;

(III) separating the extended strands of tagmented DNA and rehybridizing the transposase recognition sequences and complementary transposase recognition sequences at the ends of each of the extended strands of tagmented DNA to form complementary hairpin loops; and

(IV) sequencing the extended strand of tagmented DNA comprising complementary hairpin loops by using a sequencing polymerase.

42. The method of claim 41, wherein step (IV) is carried out in a well of an automated sequencing platform.

43. The method of claim 41, wherein prior to sequencing of step (IV):

(III') generating nanoball complexes comprising polycistronic amplified extended strands of tagmented DNA.