

CLAIMS

What is claimed is:

1. A method of preparing a DNA copy of a target polynucleotide using template switching, comprising:
mixing at least one double stranded template/primer substrate, consisting of a DNA primer oligonucleotide associated with a complementary oligonucleotide template strand, with at least one target polynucleotide in a reaction medium, and
adding a suitable amount of a non-retroviral reverse transcriptase to the reaction medium to extend the DNA primer oligonucleotide from its 3' end to provide a DNA copy polynucleotide that includes a complementary target DNA polynucleotide that is synthesized using the target polynucleotide as a template
wherein the double stranded template/primer substrate has a blunt end wherein the 3' end of the DNA primer oligonucleotide is directly aligned with the 5' end of the complementary oligonucleotide template strand, or an overhanging end wherein the 3' end of the DNA primer oligonucleotide extends 1 nucleotide beyond the 5' end of the complementary oligonucleotide template strand.
2. The method of claim 1, wherein the target polynucleotide consists of RNA.
3. The method of claim 2, wherein the target polynucleotide is a miRNA.
4. The method of claim 1, wherein the target polynucleotide consists of DNA.
5. The method of claim 1, wherein the complementary oligonucleotide template strand consists of RNA.
6. The method of claim 1, wherein the complementary oligonucleotide template strand consist of DNA.

7. The method of claim 1, wherein the double stranded template/primer substrate has an overhanging end and wherein a plurality of different double stranded template/primer substrates are used that have overhanging ends consisting of from 2-4 different nucleotides.
8. The method of claim 1, wherein the DNA primer oligonucleotide has an overhanging end and wherein the nucleotide at the 3' end of the target polynucleotide is complementary to the nucleotide at the 3' end of the DNA primer oligonucleotide.
9. The method of claim 1, wherein the non-retroviral reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide before copying the target polynucleotide to synthesize the DNA copy polynucleotide.
10. The method of claim 9, wherein the non-retroviral reverse transcriptase adds only a single additional non-complementary nucleotide.
11. The method of claim 1, wherein the 3' end of the complementary oligonucleotide template strand is terminated by a blocking agent.
12. The method of claim 1, wherein the non-retroviral reverse transcriptase is a group II intron reverse transcriptase.
13. The method of claim 1, wherein a cloning library of a plurality of DNA copy polynucleotides is prepared by using a plurality of different target polynucleotides.
14. The method of claim 1, further comprising the step of circularizing the DNA copy polynucleotide.
15. A method of preparing a DNA copy of a target polynucleotide using template switching, comprising:

mixing at least one double stranded template/primer substrate, consisting of a DNA primer oligonucleotide associated with a complementary oligonucleotide template strand, with at least one target polynucleotide in a reaction medium, and

adding a suitable amount of a group II intron reverse transcriptase to the reaction medium to extend the DNA primer oligonucleotide from its 3' end to provide a DNA copy polynucleotide that includes a complementary target DNA polynucleotide that is synthesized using the target polynucleotide as a template.

16. The method of claim 15, wherein the target polynucleotide consists of RNA.
17. The method of claim 16, wherein the target polynucleotide is a miRNA.
18. The method of claim 15, wherein the target polynucleotide consists of DNA.
19. The method of claim 15, wherein the complementary oligonucleotide template strand consists of RNA.
20. The method of claim 15, wherein the complementary oligonucleotide template strand consist of DNA.
21. The method of claim 15, wherein the double stranded template/primer substrate has a blunt end wherein the 3' end of the DNA primer oligonucleotide is directly aligned with the 5' end of the complementary oligonucleotide template strand.
22. The method of claim 15, wherein the double stranded template/primer substrate has an overhanging end wherein the 3' end of the DNA primer oligonucleotide extends 1 nucleotide beyond the 5' end of the complementary oligonucleotide template strand.
23. The method of claim 22, wherein a plurality of different double stranded template/primer substrates are used that have overhanging ends consisting of from 2-4 different nucleotides.

24. The method of claim 22, wherein the nucleotide at the 3' end of the target polynucleotide is complementary to the nucleotide at the 3' end of the DNA primer oligonucleotide.
25. The method of claim 15, wherein the reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide before copying the target polynucleotide to synthesize the DNA copy polynucleotide.
26. The method of claim 25, wherein the reverse transcriptase adds only a single additional non-complementary nucleotide.
27. The method of claim 15, wherein the 3' end of the complementary oligonucleotide template strand is terminated by a blocking agent.
28. The method of claim 15, wherein a cloning library of a plurality of DNA copy polynucleotides is prepared by using a plurality of different target polynucleotides.
29. The method of claim 15, further comprising the step of circularizing the DNA copy polynucleotide.
30. A method of adding additional nucleotides to a DNA primer oligonucleotide, comprising adding a suitable amount of a non-retroviral reverse transcriptase to a reaction medium comprising a double stranded template/primer substrate consisting of a DNA primer oligonucleotide associated with a complementary oligonucleotide template strand, wherein the non-retroviral reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide.
31. The method of claim 30, wherein the non-retroviral reverse transcriptase is a group II intron reverse transcriptase.
32. The method of claim 30, wherein 1-6 additional non-complementary nucleotides are added.

33. The method of claim 30, wherein a single non-complementary nucleotide is added.

What is claimed is:

1. A method of preparing a DNA copy of a target polynucleotide using template switching, comprising:

mixing at least one double stranded template/primer substrate, consisting of a DNA primer oligonucleotide annealed with a complementary oligonucleotide template strand, with at least one target polynucleotide in a reaction medium, and

adding a suitable amount of a non-retroviral reverse transcriptase to the reaction medium to extend the DNA primer oligonucleotide from its 3' end to provide a DNA copy polynucleotide that includes a complementary target DNA polynucleotide that is synthesized using the target polynucleotide as a template

wherein the double stranded template/primer substrate has a blunt end wherein the 3' end of the DNA primer oligonucleotide is directly aligned with the 5' end of the complementary oligonucleotide template strand, or an overhanging end wherein the 3' end of the DNA primer oligonucleotide extends 1 nucleotide beyond the 5' end of the complementary oligonucleotide template strand.

2. The method of claim 1, wherein the target polynucleotide consists of RNA.

3. The method of claim 2, wherein the target polynucleotide is a miRNA.

4. The method of claim 1, wherein the target polynucleotide consists of DNA.

5. The method of claim 1, wherein the complementary oligonucleotide template strand consists of RNA.

6. The method of claim 1, wherein the complementary oligonucleotide template strand consist of DNA.

7. The method of claim 1, wherein the double stranded template/primer substrate has an overhanging end and wherein a plurality of different double stranded template/primer substrates are used that have overhanging ends consisting of from 2-4 different nucleotides.

8. The method of claim 1, wherein the DNA primer oligonucleotide has an overhanging end and wherein the nucleotide at the 3' end of the target polynucleotide is complementary to the nucleotide at the 3' end of the DNA primer oligonucleotide.

9. The method of claim 1, wherein the non-retroviral reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide before copying the target polynucleotide to synthesize the DNA copy polynucleotide.

10. The method of claim 9, wherein the non-retroviral reverse transcriptase adds only a single additional non-complementary nucleotide.

11. The method of claim 1, wherein the 3' end of the complementary oligonucleotide template strand is terminated by a blocking agent.

12. The method of claim 1, wherein the non-retroviral reverse transcriptase is a group II intron reverse transcriptase.

13. The method of claim 1, wherein a cloning library of a plurality of DNA copy polynucleotides is prepared by using a plurality of different target polynucleotides.

14. The method of claim 1, further comprising the step of circularizing the DNA copy polynucleotide.

15. A method of preparing a DNA copy of a target polynucleotide using template switching, comprising:

mixing at least one double stranded template/primer substrate, consisting of a DNA primer oligonucleotide annealed with a complementary oligonucleotide template strand, with at least one target polynucleotide in a reaction medium, and

adding a suitable amount of a group II intron reverse transcriptase to the reaction medium to extend the DNA primer oligonucleotide from its 3' end to provide a DNA copy polynucleotide that includes a complementary target DNA polynucleotide that is synthesized using the target polynucleotide as a template.

16. The method of claim 15, wherein the target polynucleotide consists of RNA.

17. The method of claim 16, wherein the target polynucleotide is a miRNA.

18. The method of claim 15, wherein the target polynucleotide consists of DNA.

19. The method of claim 15, wherein the complementary oligonucleotide template strand consists of RNA.

20. The method of claim 15, wherein the complementary oligonucleotide template strand consist of DNA.

21. The method of claim 15, wherein the double stranded template/primer substrate has a blunt end wherein the 3' end of the DNA primer oligonucleotide is directly aligned with the 5' end of the complementary oligonucleotide template strand.

22. The method of claim 15, wherein the double stranded template/primer substrate has an overhanging end wherein the 3' end of the DNA primer oligonucleotide extends 1 nucleotide beyond the 5' end of the complementary oligonucleotide template strand.

23. The method of claim 22, wherein a plurality of different double stranded template/primer substrates are used that have overhanging ends consisting of from 2-4 different nucleotides.

24. The method of claim 22, wherein the nucleotide at the 3' end of the target polynucleotide is complementary to the nucleotide at the 3' end of the DNA primer oligonucleotide.

25. The method of claim 15, wherein the reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide before copying the target polynucleotide to synthesize the DNA copy polynucleotide.

26. The method of claim 25, wherein the reverse transcriptase adds only a single additional non-complementary nucleotide.

27. The method of claim 15, wherein the 3' end of the complementary oligonucleotide template strand is terminated by a blocking agent.

28. The method of claim 15, wherein a cloning library of a plurality of DNA copy polynucleotides is prepared by using a plurality of different target polynucleotides.

29. The method of claim 15, further comprising the step of circularizing the DNA copy polynucleotide.

30. A method of adding additional nucleotides to a DNA primer oligonucleotide, comprising adding a suitable amount of a non-retroviral reverse transcriptase to a reaction medium comprising a double stranded template/primer substrate consisting of a DNA primer oligonucleotide annealed with a complementary oligonucleotide template strand, wherein the non-retroviral reverse transcriptase adds 1-15 additional non-complementary nucleotides at the 3' end of the DNA primer oligonucleotide.

31. The method of claim 30, wherein the non-retroviral reverse transcriptase is a group II intron reverse transcriptase.

32. The method of claim 30, wherein 1-6 additional non-complementary nucleotides are added.

33. The method of claim 30, wherein a single non-complementary nucleotide is added.