

Claims

1. A composition for preparing double-stranded cDNA from RNA by an isothermal reaction comprising:
 - (a) a reverse transcriptase;
 - (b) an RNA nickase;
 - (c) a DNA polymerase with strand displacement activity or 5'-3' exonuclease activity; and
 - (d) dNTPs.
2. The composition of claim 1, wherein the activity of the reverse transcriptase is greater than the activity of the RNA nickase.
3. The composition of claim 1 or claim 2, wherein the reverse transcriptase and the RNA nickase are comprised in a single enzyme.
4. The composition of any one of claims 1-3, wherein the reverse transcriptase and the DNA polymerase are comprised in a single enzyme with both RNA-dependent and DNA-dependent polymerase activity.
5. The composition of any one of claims 1-4, wherein the DNA polymerase has strand displacement activity or the DNA polymerase has 5'-3' exonuclease activity.
6. The composition of any one of claims 1-5, wherein:
 - (a) the reverse transcriptase is a polymerase with RNA-dependent DNA polymerase activity;
 - (b) the RNA nickase is RNase H;
 - (c) the RNase H is from *Thermus thermophilus*; and/or
 - (d) the DNA polymerase is *E. coli* DNA polymerase I or *Bst* DNA polymerase.
7. The composition of claim 6, wherein the reverse transcriptase is Moloney Murine Leukemia Virus (MMLV) reverse transcriptase, a reverse transcriptase derived from a retrotransposon, or a Group II intron reverse transcriptase.
8. The composition of any one of claims 1-7, wherein the reverse transcriptase, the RNA nickase, and/or the DNA polymerase are mesophilic enzymes.

9. The composition of claim 8, wherein:
 - (a) the mesophilic enzymes have activity at 37°C - 49°C;
 - (b) the mesophilic reverse transcriptase is MMLV reverse transcriptase;
 - (c) the mesophilic RNA nickase is *E. coli* RNase H; and/or
 - (d) the mesophilic polymerase is *E. coli* DNA polymerase I.
10. The composition of any one of claims 1-7, wherein the reverse transcriptase, the RNA nickase, and/or the DNA polymerase are thermostable enzymes.
11. The composition of claim 10, wherein:
 - (a) the thermostable enzymes have activity at 50°C - 72°C;
 - (b) the thermostable reverse transcriptase is a thermostable variant of MMLV reverse transcriptase or a thermostable reverse transcriptase derived from a retrotransposon or a Group II intron reverse transcriptase;
 - (c) the thermostable RNA nickase is RNase H from *Thermus thermophilus*; and/or
 - (d) the thermostable DNA polymerase is *Bst* DNA polymerase.
12. The composition of any one of claims 1-11, wherein
 - (a) the RNA is bound to primers before preparing the double-stranded cDNA;
 - (b) the composition further comprises one or more additives chosen from DTT, BSA, Tris pH 7.5, KCl, and/or MgCl₂; and/or
 - (c) the composition has a lower units/μl of the RNA nickase as compared to the units/μl of the reverse transcriptase and/or DNA polymerase.
13. The composition of any one of claims 1-12, wherein the composition further comprises an RNA nickase inhibitor.
14. The composition of any one of claims 1-13, wherein the reverse transcriptase activity in the composition is 0.32 U/μl to 4.8 U/μl.
15. The composition of any one of claims 1-14, wherein the DNA polymerase activity in the composition is 0.04 U/μl to 0.37 U/μl.
16. The composition of any one of claims 1-15, wherein the RNA nickase activity in the composition is 0.004 U/μl to 0.04 U/μl, greater than 0.04 U/μl, or 0.05 U/μl to 0.3 U/μl.

17. A method of preparing double-stranded cDNA comprising:
 - a. combining primers with a sample comprising RNA and allowing binding of the primers to an RNA; and
 - b. combining the sample with the composition of any one of claims 1-36 and preparing double-stranded cDNA by an isothermal reaction.
18. The method of claim 17, wherein the primers comprise:
 - a. randomer primers;
 - b. primers that bind specifically to a sequence comprised in the RNA;
 - c. hexamer primers; and/or
 - d. primers comprising chemically modified nucleotides, optionally wherein the primers comprising chemically modified nucleotides render the RNA bound by the primers resistant to cleavage by the RNA nickase.
19. The method of claim 17 or 18, wherein the reverse transcriptase produces a DNA:RNA duplex comprising a first strand of cDNA and a strand of RNA.
20. The method of claim 19, wherein the RNase H nicks the RNA strand in the DNA:RNA duplex to produce RNA fragments.
21. The method of claim 20, wherein the DNA polymerase extends a second strand of DNA by priming from the RNA fragments.
22. The method of any one of claims 17-21, further comprising performing off-target RNA depletion or mRNA enrichment with the sample comprising RNA before combining primers with the sample comprising RNA.
23. The method of claim 22, wherein the mRNA enrichment comprises amplification with a poly-T primer or binding of mRNA to capture beads.