copies of RNA which were then converted back to cDNA (Phillips, et al., Methods 10(3):283-288 (1996)). This often truncates the cDNA molecule, losing 5' sequences of the original mRNA, especially for relatively long transcripts, and requires multiple rounds of processing when starting with low quantity (LQ) of cells, further exacerbating cDNA truncation. A recent modification (Hashimshony, et al., Cell Rep. 2(3):666-673 (2012)) enables multiplex analyses, but this is still 3' end sequence biased. Other methods are based on PCR amplification of cDNA (Liu, et al., Methods Enzymol. 303:45-55 (1999), Ozsolak, et al., Genome Res. 20(4):519-525 (2010), Gonzalez, et al., PLoS ONE. 5(12):e14418 (2010), Kanamori, et al., Genome Res. 21(7):1150-1159 (2011), Islam, et al., Genome Res. 21(7):1160-1167 (2011), Tang, et al., Nat. Methods. 6(5):377-382 (2009), Kurimoto, et al., Nucleic Acids Res. 34(5):e42 (2006), Qiu S, et al., Front Genet. 3:124 (2012)).

[0005] These approaches, however, may yield biased representations of sequences along the mRNA, and fail to give complete sequences for mRNAs (e.g., long mRNAs) because DNA templates (e.g., long DNA templates) are discriminated against even when a long PCR reaction is used.

SUMMARY

[0006] The present disclosure provides methods of amplifying cDNA from RNA isolated from low quantities of cells and/or single cells. The present disclosure also provides methods of transcriptome analysis where cells do not go through stress (e.g. elevated temperature for cDNA analysis), thus maintaining the transcriptome profile.

[0006a] In one aspect, the present disclosure refers to a method for preparing a complementary deoxyribonucleic acid (cDNA) molecule comprising:

(a) annealing a primer to a template nucleic acid molecule, thereby generating an annealed template nucleic acid molecule; and

(b) mixing, in the presence of nucleotides,

i. said annealed template nucleic acid molecule;

ii. one or more acceptor nucleic acid molecules; and

iii. a modified reverse transcriptase, wherein said modified reverse transcriptase:

is an R2 reverse transcriptase comprising SEQ ID NO: 50; and

generates a plurality of continuous complementary deoxyribonucleic acid molecules by: i) reverse transcribing at least a portion of said annealed template nucleic acid molecule; ii) upon reaching a 5' end of the annealed template nucleic acid, migrating to an acceptor nucleic acid molecule; and iii) reverse transcribing at least a portion of said acceptor nucleic acid molecule at a temperature of from 12° C to 42° C with an error rate of at most 5%.

[0007] In one example, the present disclosure relates to a method for preparing a complementary deoxyribonucleic acid (cDNA) molecule comprising:

CLAIMS

1. A method for preparing a complementary deoxyribonucleic acid (cDNA) molecule comprising:

(a) annealing a primer to a template nucleic acid molecule, thereby generating an annealed template nucleic acid molecule; and

(b) mixing, in the presence of nucleotides,

i. said annealed template nucleic acid molecule;

ii. one or more acceptor nucleic acid molecules; and

iii. a modified reverse transcriptase, wherein said modified reverse transcriptase:

is an R2 reverse transcriptase comprising SEQ ID NO: 50; and

generates a plurality of continuous complementary deoxyribonucleic acid molecules by: i) reverse transcribing at least a portion of said annealed template nucleic acid molecule; ii) upon reaching a 5' end of the annealed template nucleic acid, migrating to an acceptor nucleic acid molecule; and iii) reverse transcribing at least a portion of said acceptor nucleic acid molecule at a temperature of from 12° C to 42° C with an error rate of at most 5%.

2. The method of claim 1, wherein said migrating is independent of sequence identity between the template and the acceptor nucleic acid molecule.

3. The method of claim 1, wherein (a) and (b) are performed in a single vessel.

4. The method of claim 1, further comprising performing said method on a heterogeneous plurality of template nucleic acid molecules comprising a plurality of distinct ribonucleic acid (RNA) molecules.

5. The method of claim 4, wherein said plurality of distinct ribonucleic acid molecules comprise messenger RNAs (mRNAs), ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), micro RNAs (miRNAs), and long non-coding RNAs (lncRNAs).

6. The method of claim 1, wherein said plurality of continuous complementary

deoxyribonucleic acid molecules is prepared in at most 2 hours.

7. The method of claim 1, wherein said template nucleic acid molecule is selected from the group consisting of an artificially fragmented DNA template, a naturally fragmented DNA template, an artificially fragmented ribonucleic acid (RNA) template, a naturally fragmented ribonucleic acid (RNA) template, or a combination thereof.

8. The method of claim 1, wherein said modified reverse transcriptase comprises at least one improved enzyme property relative to a wild type unmodified reverse transcriptase, wherein said at least one improved enzyme property is selected from the group consisting of: higher thermostability, higher specific activity, higher processivity, higher strand displacement, higher end-to-end template jumping, higher affinity, and higher fidelity relative to said wild type unmodified reverse transcriptase.

9. The method of claim 1, further comprising adding a tag to said template nucleic acid molecule, thereby generating a plurality of tagged continuous complementary deoxyribonucleic acid molecules after performing steps (a) and (b).

10. The method of claim 9, further comprising sequencing said tagged plurality of continuous complementary deoxyribonucleic acid molecules.

11. The method of claim 1, wherein said modified reverse transcriptase further comprises a tag.

12. The method of claim 11, wherein said tag is selected from the group consisting of biotin, azido group, acetylene group, His-tag, calmodulin-tag, CBP, CYD, Strep II, FLAG-tag, HA-tag, Myc-tag, S-tag, SBP-tag, Softag-1, Softag-3, V5-tag, Xpress-tag, isopeptag, SpyTag B, HPC peptide tags, GST, MBP, biotin carboxyl carrier protein, glutathione-S-transferase-tag, green fluorescent protein-tag, maltose binding protein-tag, Nus-tag, Strep-tag, and thioredoxin-tag.

13. The method of claim 1, further comprising performing a polymerase chain reaction (PCR) amplification reaction, thereby forming one or more amplicons.

14. The method of claim 13, wherein (a) and (b) and said PCR amplification reaction are performed in the same vessel.

15. The method of claim 13, wherein said PCR amplification is performed at a temperature sufficient to inactivate the reverse transcriptase.

16. The method of claim 1, wherein said one or more acceptor nucleic acid molecules comprises a modified nucleotide that stops the reverse transcription by said modified reverse transcriptase.

17. The method of claim 1, further comprising annealing one or more primers to one or more template nucleic acid molecules in a biological sample obtained from a subject.

18. The method of claim 17, wherein said sample is a tissue sample.

19. The method of claim 17, wherein said sample comprises one or more cell free nucleic acids and the method further comprises performing the reaction of (a) and (b) in the same vessel.

20. The method of claim 17, further comprising depleting at least one ribosomal RNA (rRNA) from said sample comprising one or more template nucleic acid molecules prior to annealing one or more primers.

21. The method of claim 20, wherein the step for depleting said at least one ribosomal RNA (rRNA) comprises hybridization of an oligonucleotide to said rRNA.

22. The method of claim 20, wherein the step for depleting said at least one ribosomal RNA (rRNA) comprises an oligonucleotide probe-guided endonucleolytic cleavage of said rRNA.

23. The method of claim 17, wherein said one or more template nucleic acid molecules comprise at least one transfer RNA (tRNA) and said method further comprises depleting said at least one tRNA from said one or more template nucleic acid molecules prior to annealing one or more primers.

24. The method of claim 1, wherein said method is performed in the absence of purification of said one or more annealed template nucleic acid molecules.

25. The method of claim 1, further comprising purifying said mixture comprising said plurality of continuous complementary deoxyribonucleic acid molecule.

26. The method of claim 25, wherein said purifying comprises two purification steps.

27. The method of claim 26, wherein said two purification steps comprise a nickel and a heparin affinity purification steps.

28. The method of claim 1, wherein said method is performed on from 0.1 nM to 100 nM of said one or more template nucleic acid molecules.

29. The method of claim 1, further comprising annealing one or more random primer(s) to said template nucleic acid molecule.

30. The method of claim 1, wherein said primer hybridizes to one or more adapter sequence.

31. The method of claim 1, wherein said template nucleic acid molecule is derived from a single cell.