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 a sequence selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, and SEQ ID NO: 67; 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) 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:

(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 generates a plurality of continuous complementary deoxyribonucleic acid molecules by: i) reverse transcribing a sequence of said annealed template nucleic acid molecule; ii) migrating to an acceptor nucleic acid molecule; and iii) reverse transcribing a sequence of said acceptor nucleic acid molecule at a temperature of from about 12 ºC to about 42 ºC with an error rate of at most about 5%.

[0008] In some examples, the migrating is independent of sequence identity between the template and the acceptor nucleic acid molecule. In some examples, (a) and (b) are performed in a single vessel. In some examples, the method further comprises performing the method on a heteregoneous plurality of template nucleic acid molecules comprising a plurality of distinct ribonucleic acid (RNA) molecules. In some examples, the 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). In some examples, the plurality of continuous complementary deoxyribonucleic acid molecules is prepared in at most about 2 hours. In some examples, the 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.

[0009] In some examples, the modified reverse transcriptase amplifies said annealed template nucleic acid molecule at a processivity of at least about 80% per base. In some examples, the modified reverse transcriptase comprises at least one improved enzyme property relative to a wild type unmodified reverse transcriptase. In some examples, at least one improved enzyme property is selected from the group consisting of: higher thermo-stability, 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. In some examples, modified reverse transcriptase is an R2 reverse transcriptase. In some examples, the modified reverse transcriptase has at least about 90% identity to SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67, and contains at least one substitution modification relative to SEQ ID NO: 52.

[0010] In some examples, the method further comprises adding a tag to the template nucleic acid molecule, thereby generating a plurality of tagged continuous complementary deoxyribonucleic acid molecules after performing (a) and (b). In some examples, the method further comprises sequencing the

tagged plurality of continuous complementary deoxyribonucleic acid molecules. In some examples, modified reverse transcriptase further comprises a tag. In some examples, the 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.

[0011] In some examples, the method further comprises performing a polymerase chain reaction (PCR) amplification reaction, thereby forming one or more amplicons. In some examples, (a) and (b) and the PCR amplification reaction are performed in the same vessel. In some examples, the PCR amplification is performed at a temperature sufficient to inactivate the reverse transcriptase.

[0012] In some examples, the one or more acceptor nucleic acid molecules comprise a modified nucleotide that stops the reverse transcription by the modified reverse transcriptase. In some examples, the method further comprises obtaining a sample comprising one or more template nucleic acid molecules from a subject and annealing one or more primers to said one or more template nucleic acid molecules. In some examples, the sample is a tissue sample. In some examples, the 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.

[0013] In some examples, the method further comprises depleting at least one ribosomal RNA (rRNA) from the sample comprising one or more template nucleic acid molecules prior to annealing one or more primers. In some examples, depleting the at least one ribosomal RNA (rRNA) comprises hybridization of an oligonucleotide to the rRNA. In some examples, depleting said at least one ribosomal RNA (rRNA) comprises an oligonucleotide probe-guided endonucleolitic cleavage of the rRNA. In some examples, the one or more template nucleic acid molecules comprise at least one transfer RNA (tRNA) and the method further comprises depleting the at least one tRNA from the one or more template nucleic acid molecules prior to annealing one or more primers.

[0014] In some examples, the method is performed in the absence of purification of one or more annealed template nucleic acid molecules. In some examples, the method further comprises purifying the mixture comprising a plurality of continuous complementary deoxyribonucleic acid molecule. In some examples, purifying comprises two purification steps. In some examples, the two purification steps comprise a nickel and a heparin affinity purification steps.

[0015] In some examples the method is performed on from about 0.1 nM to about 100 nM of one or more template nucleic acid molecules. In some examples, the method further comprises annealing one or more random primer(s) to the template nucleic acid molecule. In some examples, a primer hybridizes to one or more adapter sequence. In some examples, the template nucleic acid molecule is derived from a single cell.

[0016] In one examples, the present disclosure relates to a method for preparing a complementary deoxyribonucleic acid molecule comprising:

(a) annealing one or more primers to an amount of template nucleic acid molecules, thereby

generating one or more annealed template nucleic acid molecules; and

(b) mixing, in the presence of nucleotides,

i. said one or more annealed template nucleic acid molecules;

ii. one or more acceptor nucleic acid molecules; and

iii. a modified reverse transcriptase, whereby said modified reverse transcriptase generates a plurality of continuous complementary deoxyribonucleic acid molecule by reverse transcribing a sequence of an annealed template nucleic acid molecule, migrating to an acceptor nucleic acid molecule, and reverse transcribing a sequence of said acceptor nucleic acid molecule without thermal cycling in a single reaction vessel.

[0017] In some examples, the migrating is independent of sequence identity between the template and the acceptor nucleic acid molecule. In some examples, the plurality of continuous complementary deoxyribonucleic acid molecules are prepared in at most about 2 hours. In some examples, the amount of template nucleic acid molecules 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. In some examples, the amount of template nucleic acid molecules comprises one or more cell free nucleic acids. In some examples, the modified reverse transcriptase comprises at least one improved enzyme property relative to a wild type unmodified reverse transcriptase. In some examples, the improved enzyme property is selected from the group consisting of: higher thermo-stability, 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. In some examples, the modified reverse transcriptase is an R2 reverse transcriptase. In some examples, the modified reverse transcriptase has at least 90% identity to SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 and contains at least one substitution modification relative to SEQ ID NO: 52.

[0018] In some examples, the method further comprises adding a tag to the amount of template nucleic acid molecules, thereby generating a plurality of tagged continuous complementary deoxyribonucleic acid molecules after performing (a) and (b). In some examples, the method further comprises sequencing the plurality of tagged continuous complementary deoxyribonucleic acid molecules. In some examples, the modified reverse transcriptase comprises a tag. In some examples, the 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. In some examples, the one or more acceptor nucleic acid molecules comprises a

modified nucleotide that stops the reverse transcription by said modified reverse transcriptase.

[0019] In some examples, the method further comprises obtaining a sample from a subject. In some examples, the 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. In some examples, the sample is a tissue sample. In some examples, the method further comprises depleting at least one ribosomal RNA (rRNA) from the one or more template nucleic acid molecules prior to annealing one or more primers. In some examples, depleting said at least one ribosomal RNA (rRNA) comprises hybridization of an oligonucleotide to the rRNA. In some examples, depleting the at least one ribosomal RNA (rRNA) comprises an oligonucleotide probe-guided endonucleolitic cleavage of the rRNA. In some examples, depleting at least one transfer RNA (tRNA) from the amount of template nucleic acid molecules is prior to annealing one or more primers.

[0020] In some examples, the method is performed in the absence of purification of one or more annealed template nucleic acid molecules. In some examples the method further comprises purifying the mixture comprising the plurality of continuous complementary deoxyribonucleic acid molecules. In some examples, purifying comprises two purification steps. In some examples, the two purification steps comprise a nickel and a heparin affinity purification steps. In some examples, the amount of template nucleic acid molecules is from about 0.1 nM to about 100 nM of said one or more template nucleic acid molecules. In some examples, the one or more primers comprise one or more random primer(s). In some examples, the one or more primers is hybridized to one or more adapter sequence. In some examples, the amount of template nucleic acid molecules are derived from a single cell.

[0021] In one example, the present disclosure relates to a polypeptide having reverse transcriptase activity comprising an amino acid sequence that has at least 90% identity to SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67 and contains at least one substitution modification relative to SEQ ID NO: 52.

[0022] In one example, the present disclosure relates to a purified polypeptide comprising one or more domains from an R2 reverse transcriptase wherein said purified polypeptide generates one or more copies of a complementary deoxyribonucleic acid molecule from a template nucleic acid molecule with an error rate of at most about 5%.

[0023] In some examples, the polypeptide reverse transcribes a template nucleic acid molecule at a processivity of at least about 80% per base. In some examples, the polypeptide reverse transcribes a template nucleic acid molecule at a temperature of from about 12 ^{o}C to about 42 ^{o}C. In some examples, the polypeptide comprises an amino acid sequence as set forth in SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67.

[0024] In one example, the present disclosure relates to a non-naturally occurring enzyme comprising

one or more domains from an R2 reverse transcriptase wherein the non-naturally occurring enzyme generates a complementary deoxyribonucleic acid product in a time period of less than about three hours and at a performance index greater than 1.0 for at least one enzyme property selected from the group consisting of improved stability, specific activity, protein expression, purification, processivity, strand displacement, template jumping, increased DNA/RNA affinity, and fidelity, as compared to a purified enzyme of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, or SEQ ID NO: 67.

[0025] In some examples, an R2 reverse transcriptase and/or a non-naturally occurring enzyme and/or a polypeptide can have at least one mutation and/or modification. In some examples, an R2 and/or a non-naturally occurring enzyme and/or a polypeptide comprises a C952S, and/or C956S, and/or C952S, C956S (double mutant), and/or C969S, and/or H970Y, and/or R979Q, and/or R976Q, and/or R1071S, and/or R328A, and/or R329A, and/or Q336A, and/or R328A, R329A, Q336A (triple mutant), and/or G426A, and/or D428A, and/or G426A,D428A (double mutant) mutation, and/or any combination thereof.

[0026] In one example, the present disclosure relates to a method comprising:

(a) adding a nuclease complex to a plurality of double-stranded nucleic acid molecules, wherein said nuclease complex comprises:
   i. a Cas9 nuclease or a functional variant thereof; and
   ii. at least one synthetic guide oligonucleotide, wherein said synthetic guide oligonucleotide is complementary to a ribosomal ribonucleic acid (rRNA) or transfer ribonucleic acid (tRNA) region in at least one double-stranded nucleic acid molecule in said plurality of double-stranded nucleic acid molecules;

(b) permitting said complex to cleave said rRNA or tRNA region of at least one double-stranded nucleic acid molecule, thereby providing at least one cleaved double-stranded nucleic acid molecule, and

(c) subjecting said at least one cleaved double-stranded nucleic acid molecule or derivative thereof to nucleic acid sequencing, thereby yielding a nucleic acid sequence of said at least one double-stranded nucleic acid molecule lacking said rRNA or tRNA region.

[0027] In some examples, the synthetic guide oligonucleotide is complementary to a ribosomal ribonucleic acid (rRNA). In some examples, the synthetic guide oligonucleotide is complementary to a transfer ribonucleic acid (tRNA). In some examples, the method does not require denaturation of the plurality of double-stranded molecules comprising sequences derived from rRNAs and tRNAs prior to sequencing. In some examples, the plurality of double-stranded nucleic acid molecules comprise a cDNA.

[0028] In some examples, the method does not require denaturation of the plurality of double-stranded molecules comprising sequences derived from rRNAs and/or tRNAs. In some examples, the

plurality of double-stranded nucleic acid molecules comprises sequences derived from an rRNA and/or a tRNA. In some examples, the double-stranded nucleic acid molecules comprises cDNA. In some examples, the pre-determined region is a region of an rRNA. In some examples, the pre-determined region is a region of a tRNA.

[0029] In one example, the present disclosure relates to a method for preparing a concatemer of nucleic acid molecules comprising:

(a) processing ends of a plurality of double-stranded nucleic acid molecules;

(b) adding a first plurality of adaptor molecules to said plurality of double stranded nucleic acid molecules, wherein said first plurality of adaptor molecules comprise one or more overhang sequences, wherein at least two overhang sequences are complentary to each other, thereby providing a first plurality of adaptor connected double-stranded nucleic acid molecules;

(c) adding a polymerizing enzyme to said first plurality of adaptor connected double-stranded nucleic acid molecules in the absence of a primer, whereby said polymerizing enzyme forms a first set of adaptor connected double-stranded nucleic acid concatemers by joining two or more adaptor connected double-stranded nucleic acid molecules by said one or more overhang sequences;

(d) adding a second plurality of adaptor molecules to said first set, wherein said second plurality of adaptor molecules comprise one or more overhang sequences, wherein at least two overhang sequences are complementary to each other, thereby providing a second set of adaptor connected double-stranded nucleic acid molecules;

(e) repeating (a)-(c) with a set of adaptor molecules to yield a concatemer comprising a predetermined average length.

[0030] In some examples, the processing of (a) comprises end repair. In some examples, the polymerizing enzyme is a reverse transcriptase. In some examples, the reverse transcriptase is an R2 reverse transcriptase or a functional variant thereof. In some examples, the first plurality of adaptor molecules, the second plurality of adaptor molecules, or both comprise a unique molecular identifier sequence (UMI). In some examples, the polymerizing enzyme joins two or more adaptor connected double-stranded nucleic acid molecules in a PCR or isothermal amplification reaction. In some examples, the first plurality of adaptor molecules, the second plurality of adaptor molecules, or both comprise at least one modified nucleotide

[0031] In some examples, the adaptor comprises a unique molecular identifier sequence (UMI). In some examples, the amplifying is performed by PCR or isothermal amplification. In some examples, the adaptor comprises at least one modified nucleotide.

[0032] Advantages of the present disclosure include, but is not limited to: efficient and simple method for cDNA and nucleic acid library preparation (e.g., single cell and/or bulk library preparation) that is compatible with various sequence technologies; high quality library preparation that can be used for single cell nucleic acid (e.g., RNA) sequencing and bulk nucleic acid (e.g., RNA) sequencing;

modified reverse transcriptase enzymes with improved enzyme property; high conversion (e.g., efficiency and/or fidelity) of a nucleic acid sample (e.g., RNA, and/or mRNA, and/or DNA) to nucleic acid (e.g., cDNA) library; low non-specific products yield; and transcriptome analysis where cells don't go through stress (e.g., no need for elevated temperature for cDNA synthesis) because nucleic acid synthesis may be performed at ambient temperatures (e.g., 30 °C).

[0033] Advantages of the present disclosure also include the ability to produce a nucleic acid (e.g., cDNA) library using random or multi-priming and/or a library from fragmented/degraded nucleic acid molecule(s) (e.g., RNA and DNA), even at low amounts (e.g., 500 femtomolar) of fragmented/degraded nucleic acid molecule(s). Advantages of the present disclosure also include the ability of the disclosed methods (e.g., by using a modified reverse transcriptase) to amplify a fragment (e.g., amplification of the full fragment) and generate multiple copies of the full fragment for sequencing. Current available methods may cause a fragment to be amplified at a random location and may only amplify sections of the fragment, thus sequencing and identification of the fragment(s) according to current methods is not available at low amounts. Advantages of the present disclosure also include amplification via a single step or via a two step amplification protocol, thus increasing specificity and efficiency. Current methods include multiple step amplification that can result in low yield, low efficiency, and/or low specificity. Another adavantage of the methods of the present disclosure is the capability to prime using a piece of nucleic acid molecule (e.g., RNA) that does not have to be complementary to a template. Another advantage of the present disclosure is the ability of the modified enzymes to template jump at room temperature and/or at temperatures as low as about 30 °C. Another advantage of the present disclosure is the ability to prepare a library from a sample in less than three steps (Figure 1) and/or less than 4 hours. This advantage is particular important for clinical research and testing and the medical field. Another advantage of the present disclosure is the ability of the methods disclosed herein (e.g., by using a modified reverse transcriptase) to improve template jumping, processivity, strand displacement properties, enzyme activity, and/or fidelity.

[0034] Additional aspects and advantages of the present disclosure will become readily apparent to those skilled in this art from the following detailed description, wherein only illustrative examples of the present disclosure are shown and described. As will be realized, the present disclosure is capable of other and different examples, and its several details are capable of modifications in various obvious respects, all without departing from the disclosure. Accordingly, the drawings and description are to be regarded as illustrative in nature, and not as restrictive.

INCORPORATION BY REFERENCE

[0035] All publications, patents, and patent applications, and NCBI accession numbers mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, patent application, or NCBI accession number was specifically and individually

indicated to be incorporated by reference. To the extent publications and patents, patent applications, or NCBI accession numbers incorporated by reference contradict the disclosure contained in the specification, the specification is intended to supersede and/or take precedence over any such contradictory material.

BRIEF DESCRIPTION OF THE DRAWINGS

[0036] The novel features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also "Figure" and "FIG."

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 a sequence selected from the group consisting of SEQ ID NO: 49, SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61, SEQ ID NO: 62, SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, and SEQ ID NO: 67; 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) 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 thermos-stability, 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.