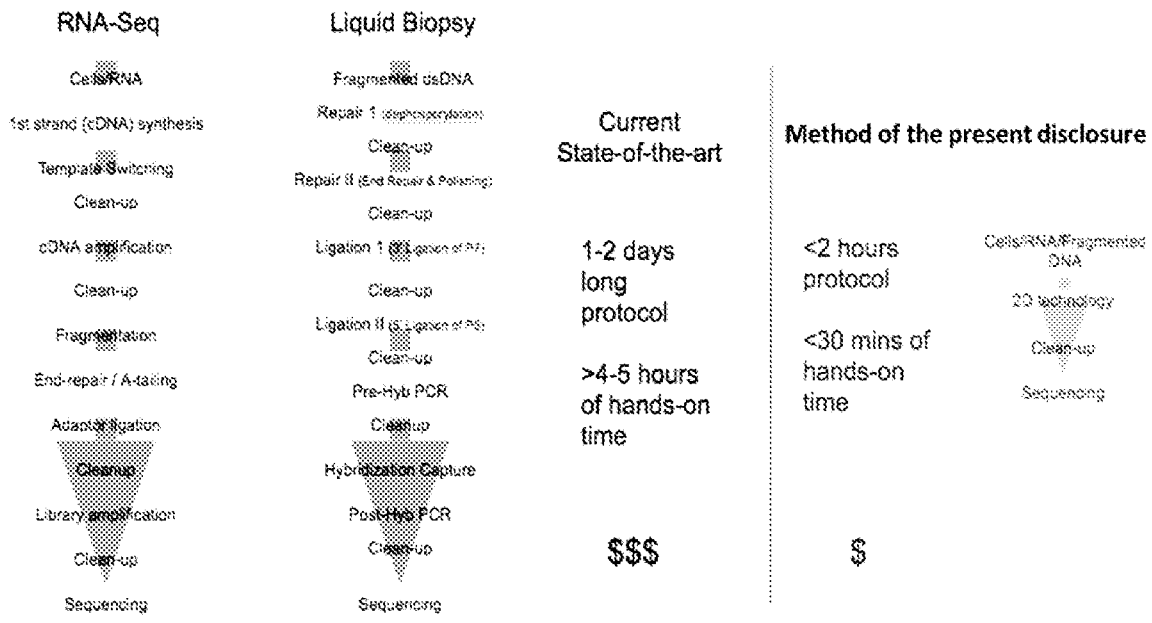


FIGURE 1

Workflow comparison

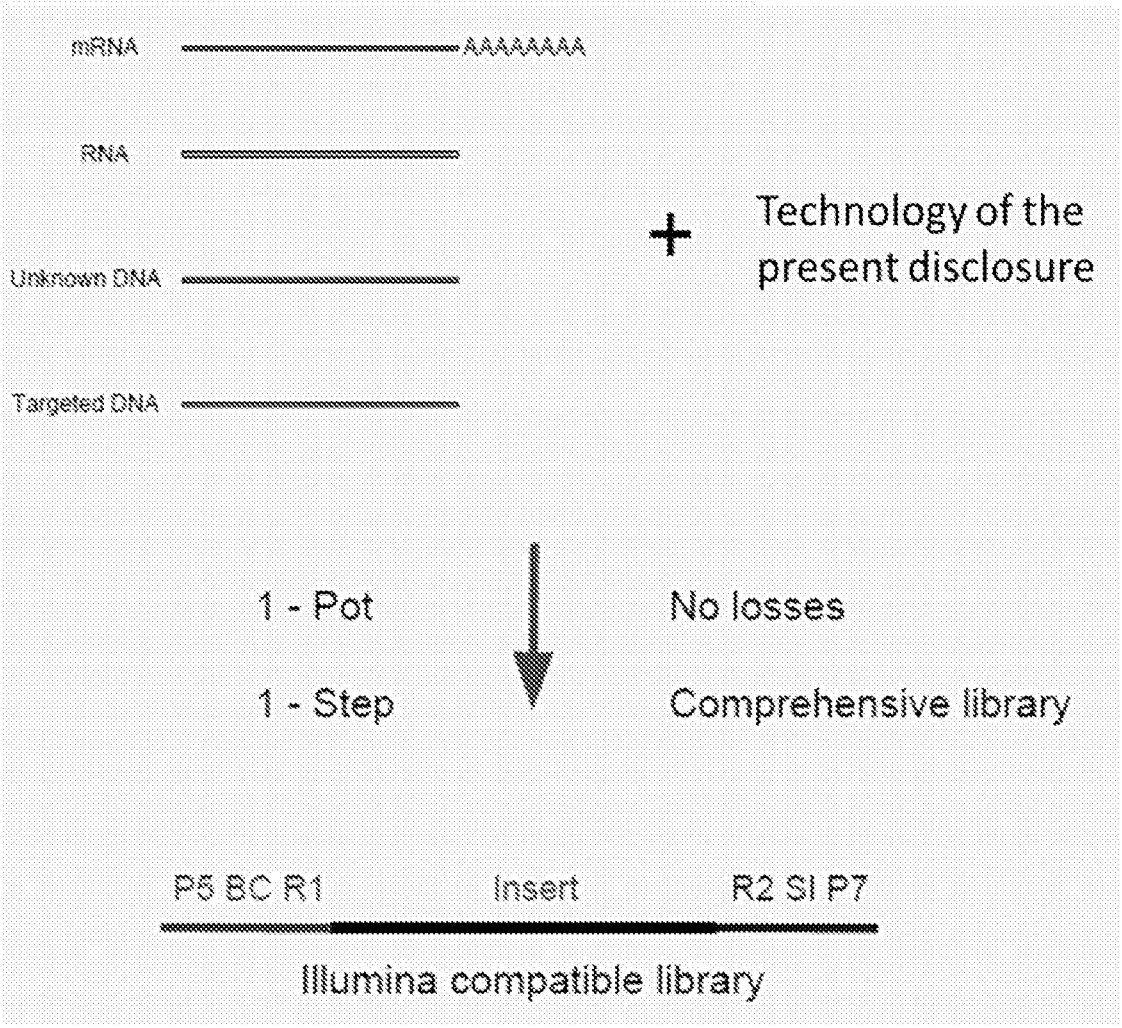


FIGURE 2

Example of a workflow of the present disclosure for constructing a library for sequencing

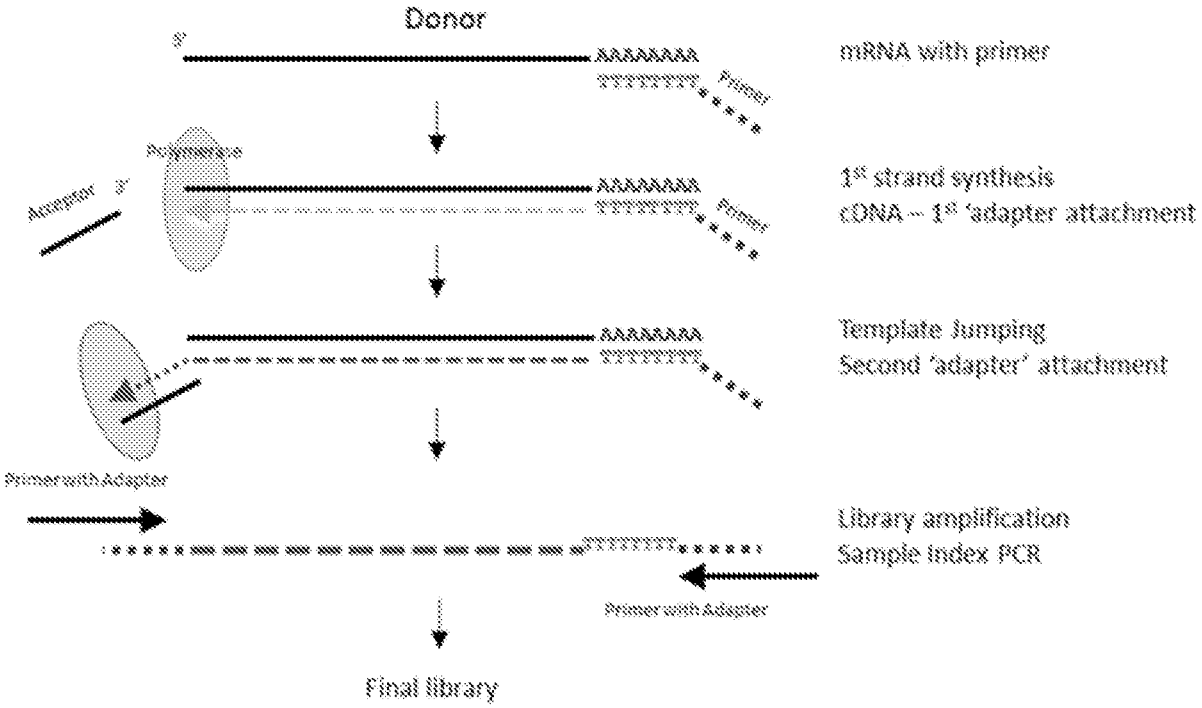


FIGURE 3

Example of a workflow for constructing a library

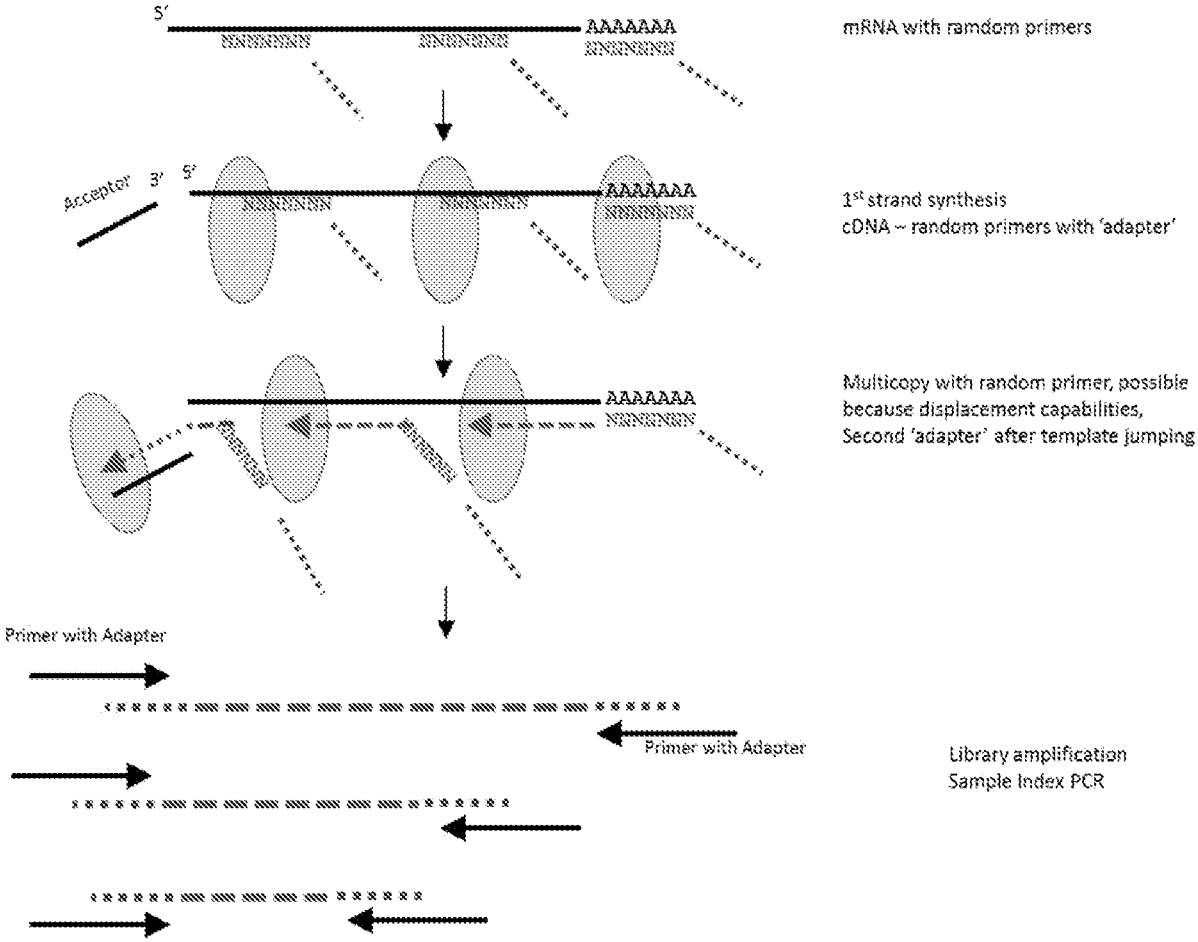


FIGURE 4

Example of a workflow using random primers for constructing a library

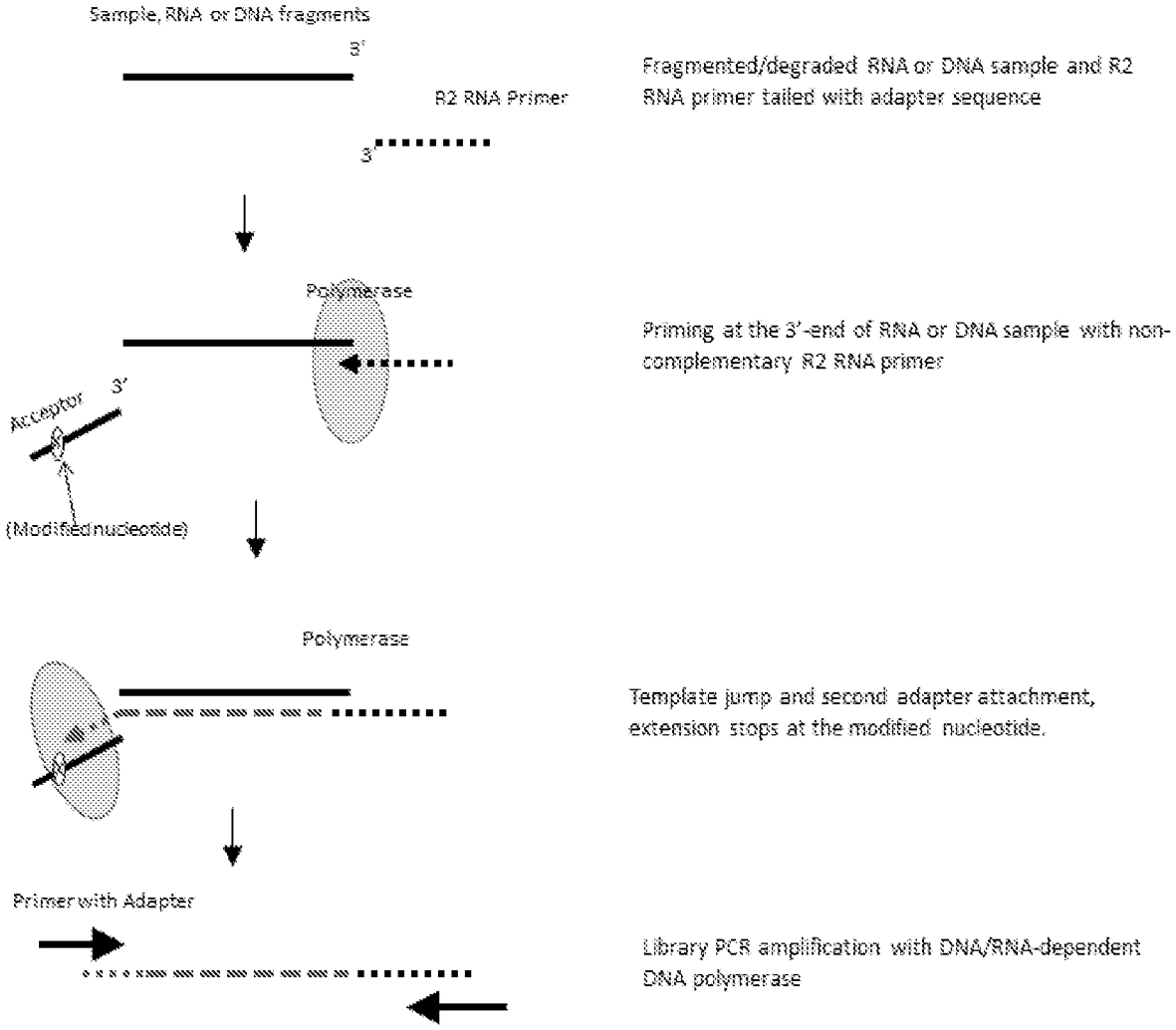


FIGURE 5A

Example of a workflow using fragmented or degraded RNA or DNA with RNA priming for constructing a library

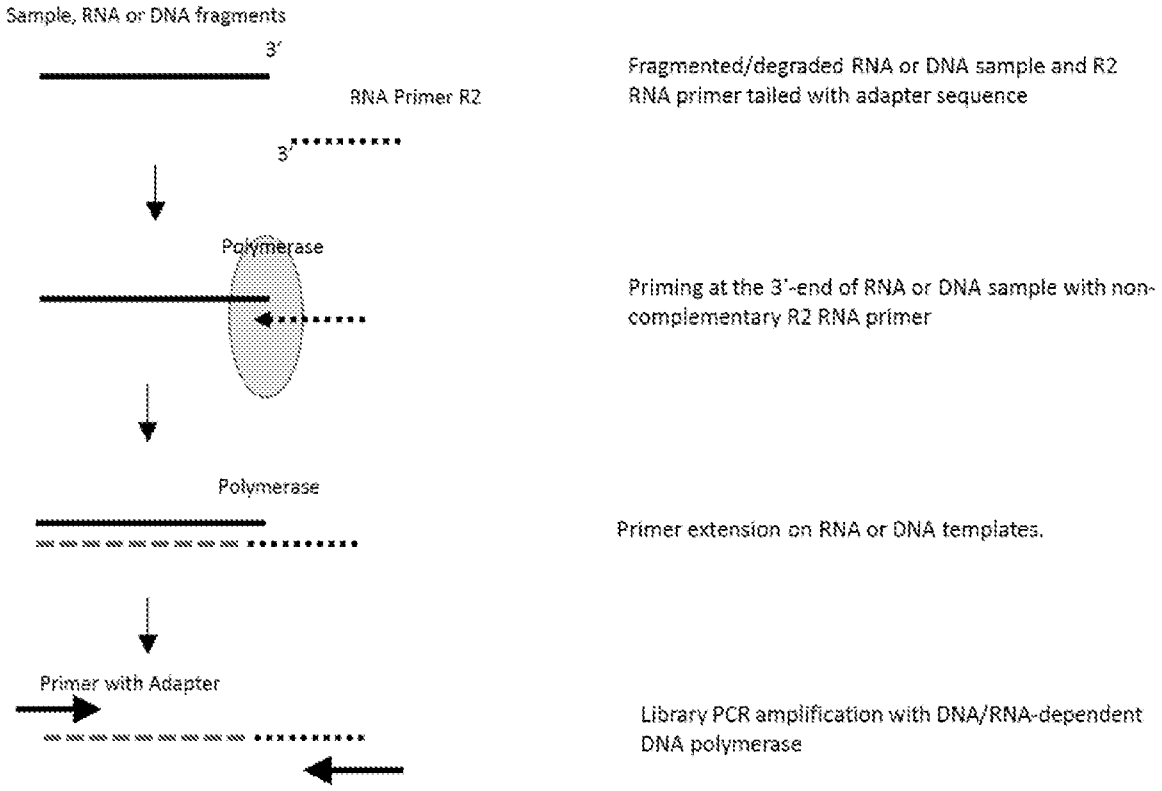


FIGURE 5B

Example of a workflow using fragmented or degraded RNA or DNA with RNA priming for constructing a library – method with specific primer

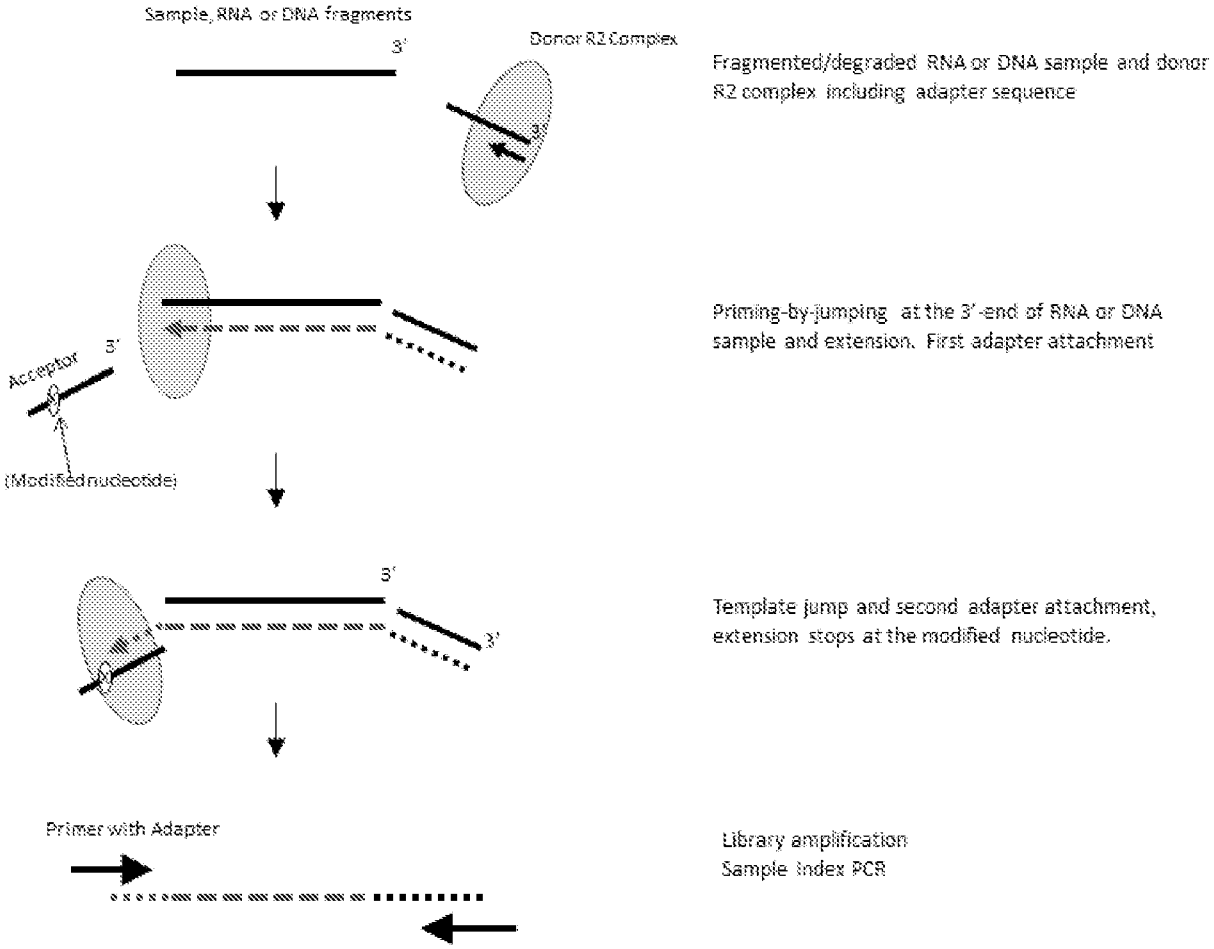


FIGURE 6A

Example of a workflow using fragmented or degraded RNA or DNA with a donor complex for constructing a library

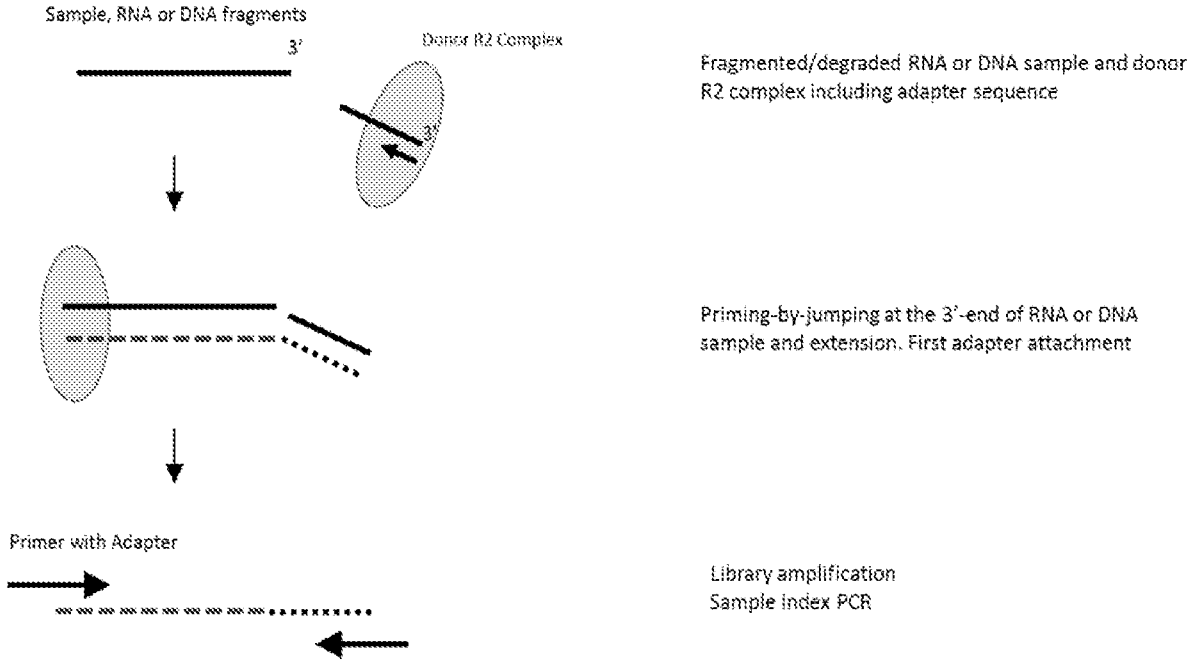


FIGURE 6B

Example of a workflow using fragmented or degraded RNA or DNA with a donor complex for constructing a library – with specific primer

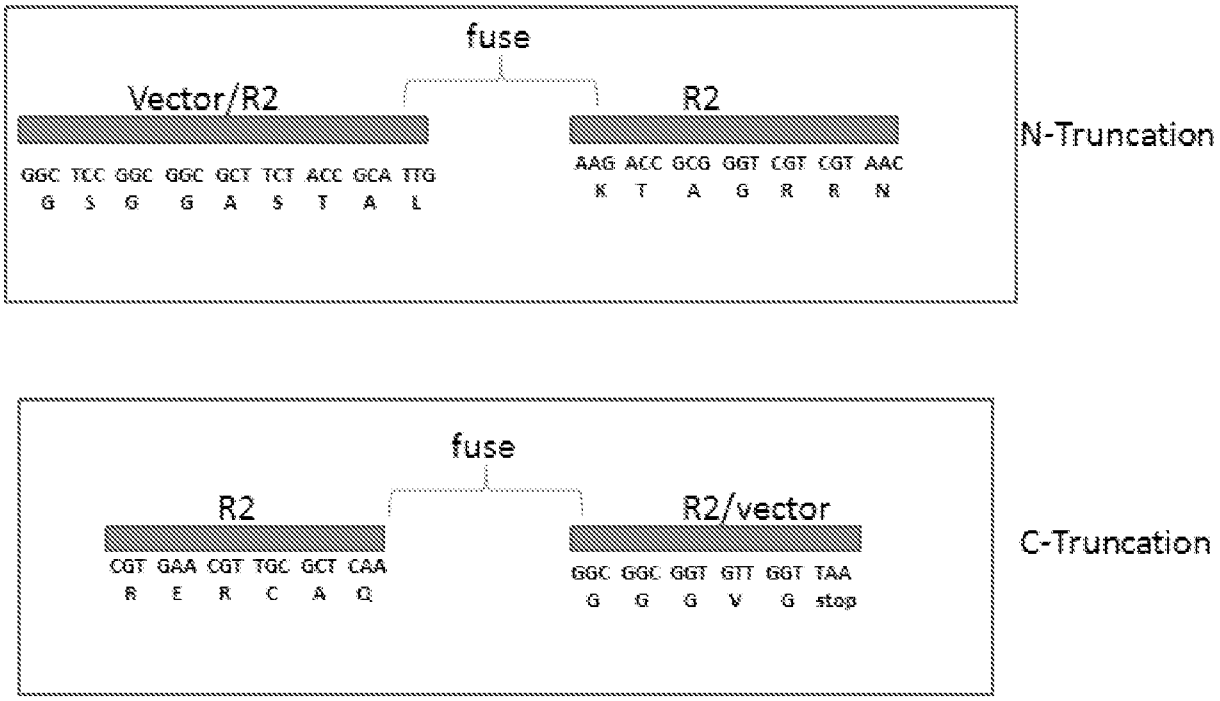


FIGURE 7

Schematic representation of an N-terminal and a C-terminal truncation of an R2 reverse transcriptase

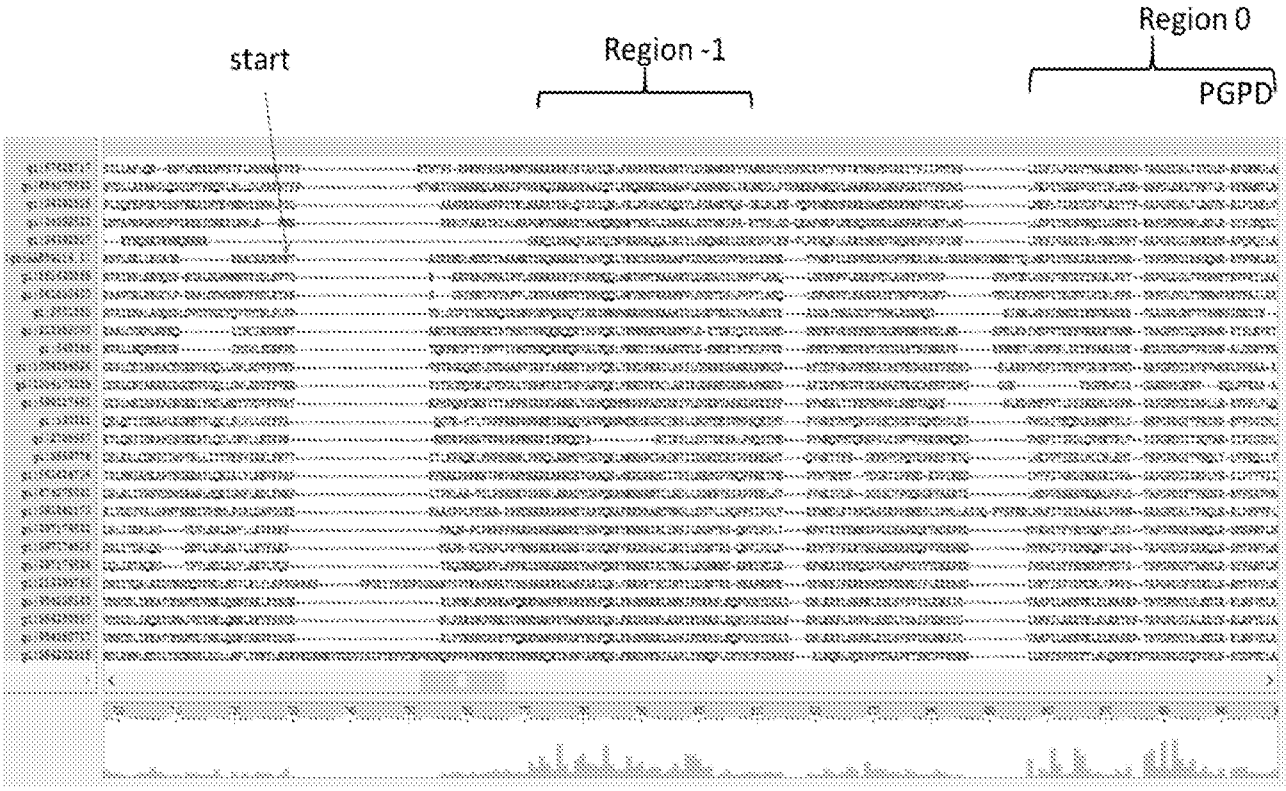


FIGURE 8

Sequence analysis with selected non-long terminal repeat (LTR) retrotransposon and an example of a site of N-terminal truncation upstream two conservative regions (region -1 and region 0)

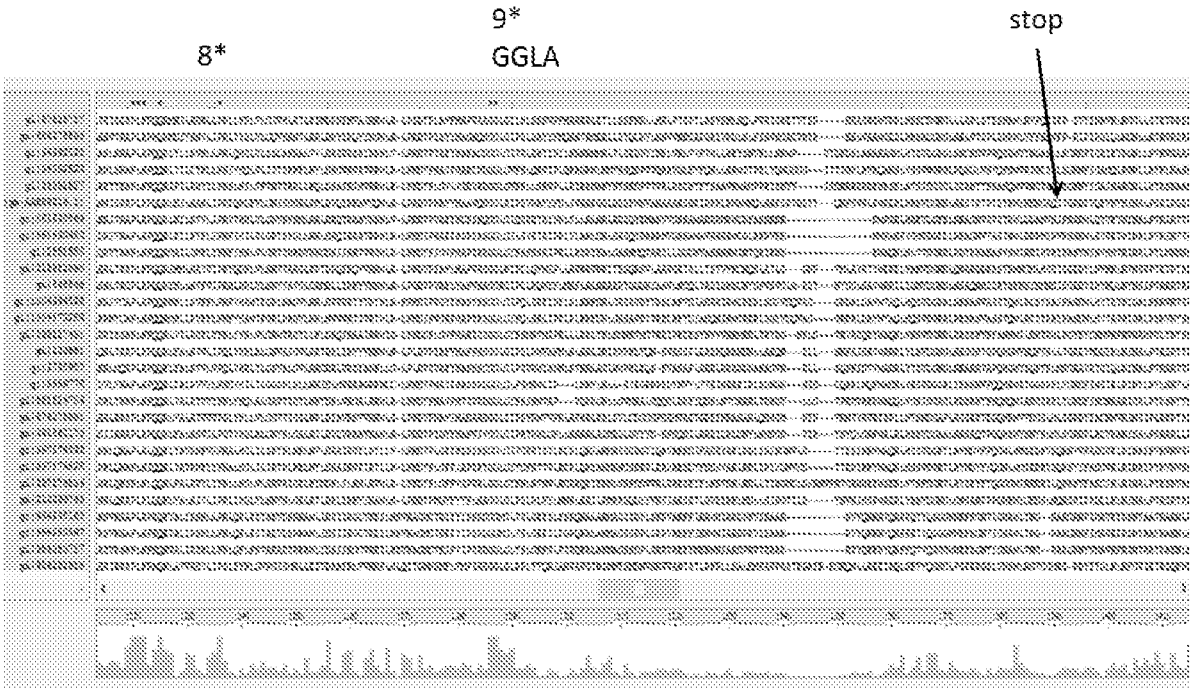


FIGURE 9

Sequence analysis with selected non-LTR retrotransposon and an example of a site of C-truncation downstream two conservative motifs 8* and 9*

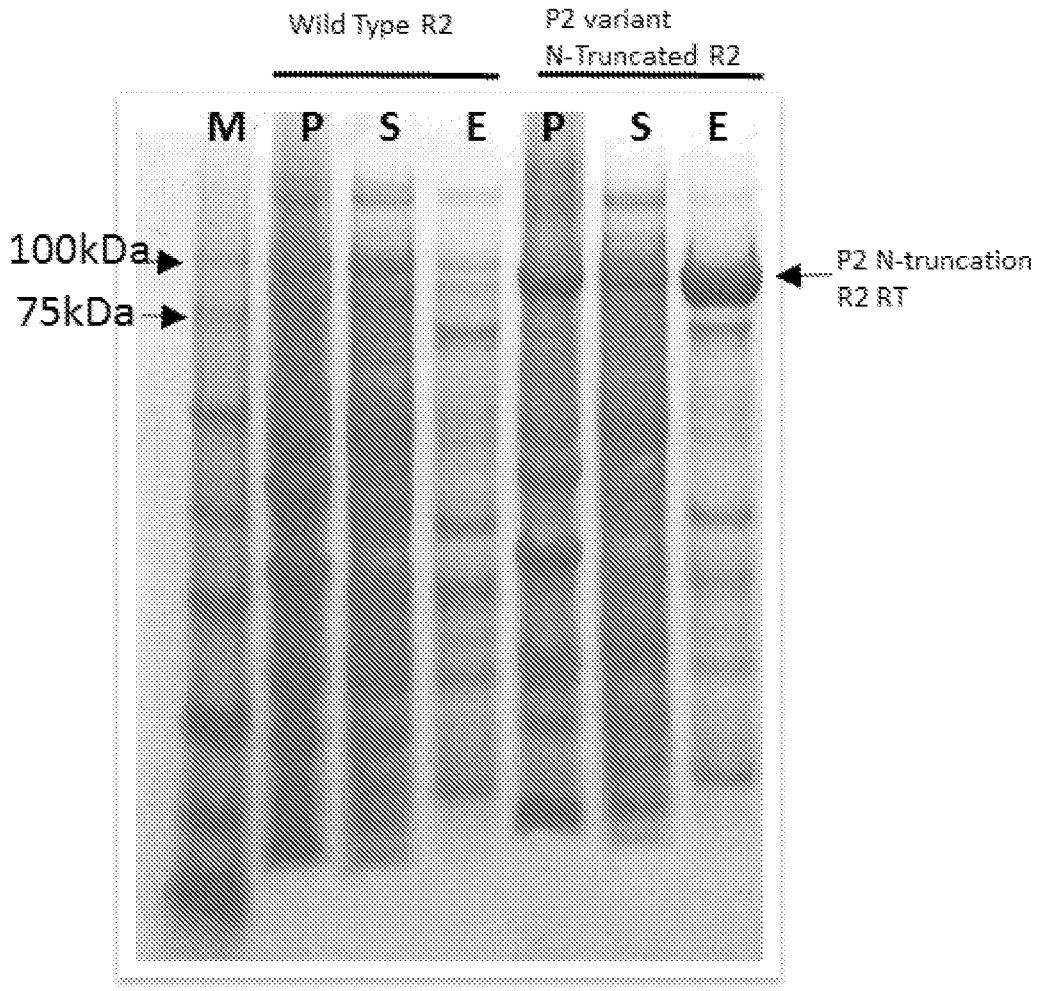


FIGURE 10

Purification of a wild type R2 reverse transcriptase and an N-terminal truncated R2 reverse transcriptase

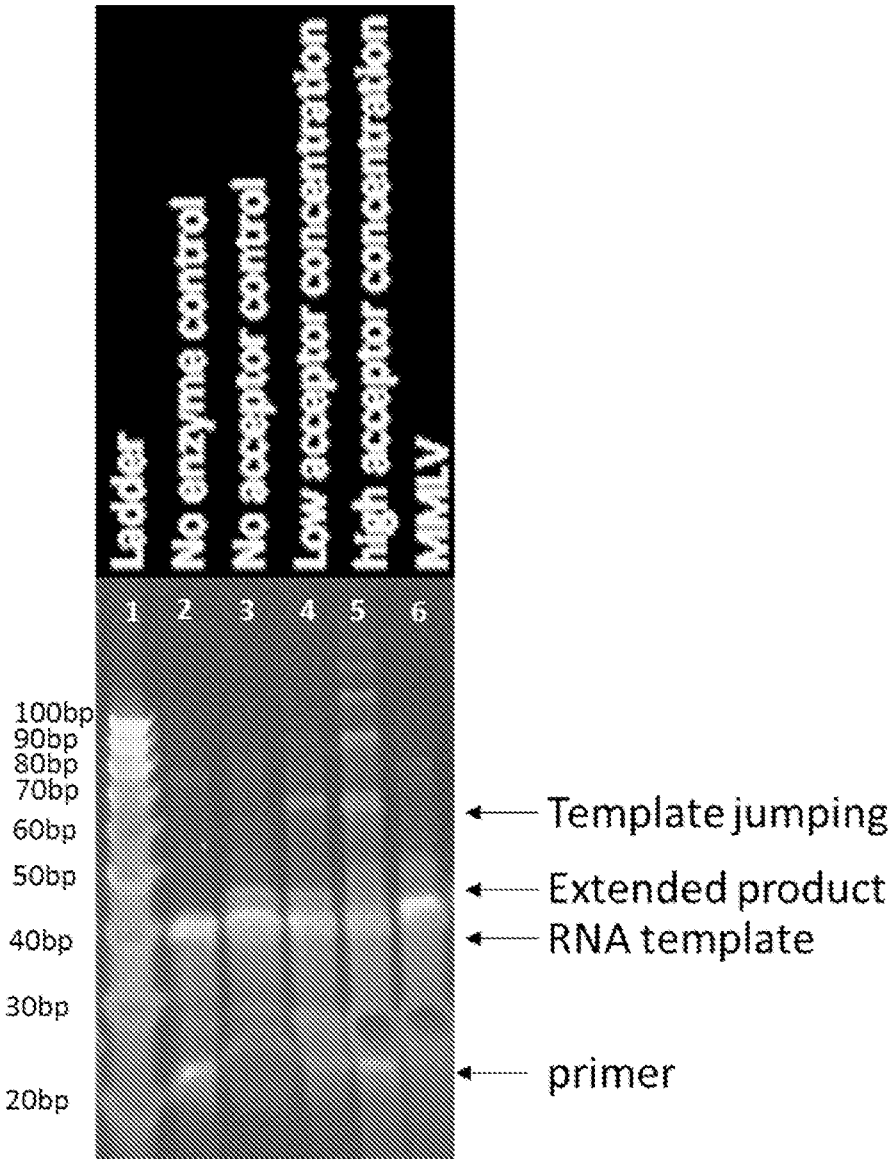


FIGURE 11

PAGE gel showing activity and template jumping properties of R2 enzyme and MMLV using synthetic RNA

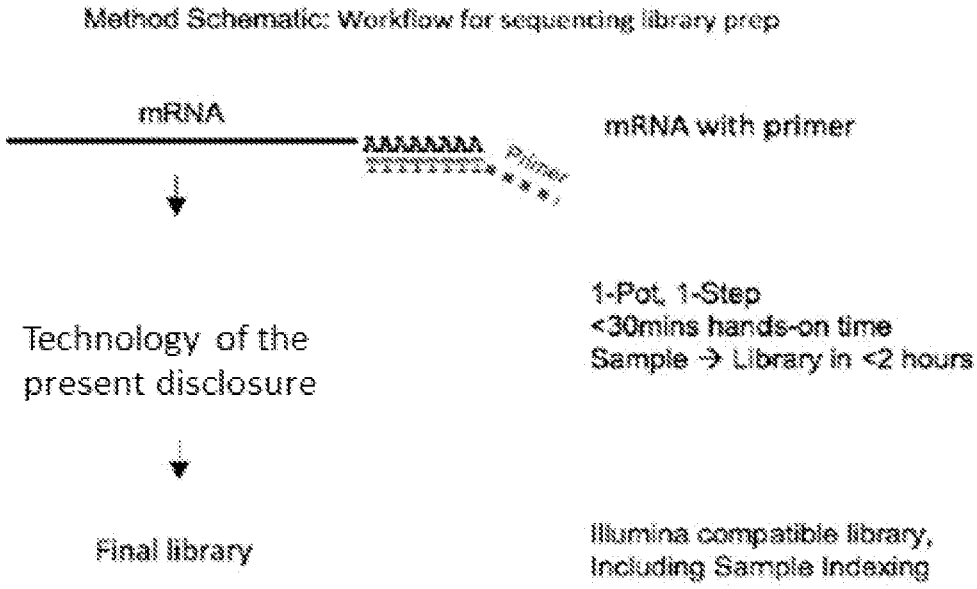


FIGURE 12A

Workflow for sequencing library preparation

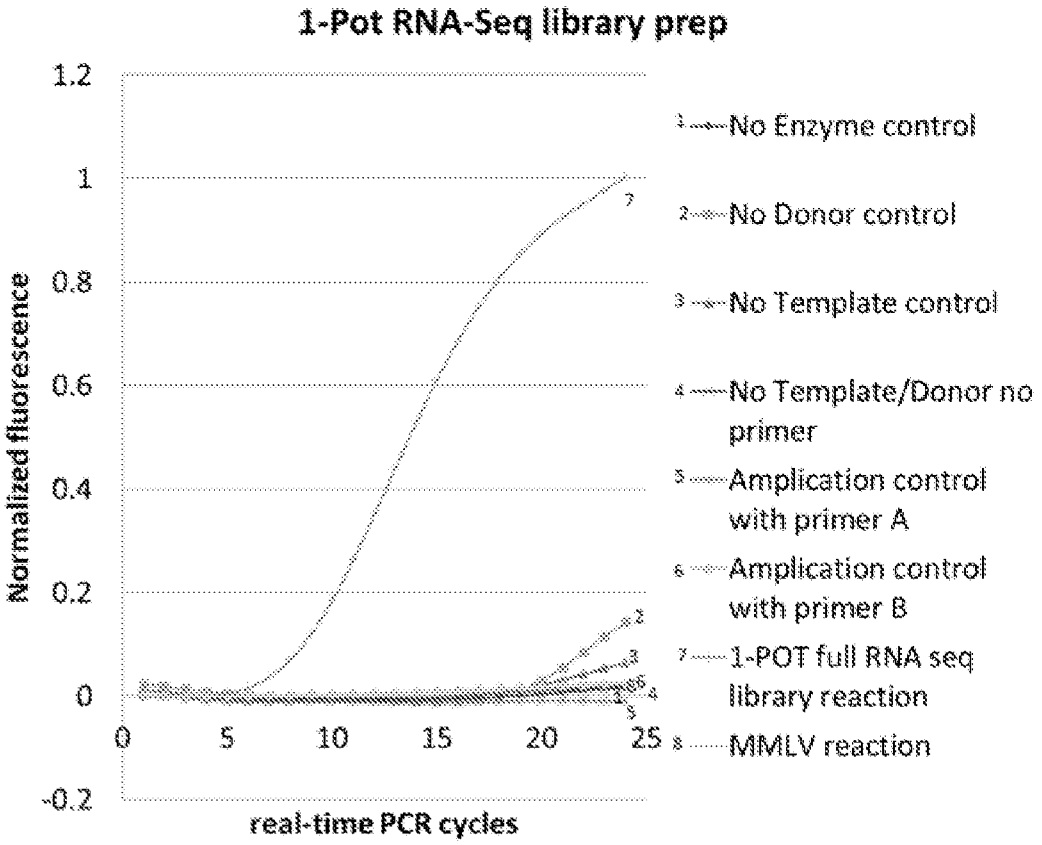


FIGURE 12B

Real-time PCR data of library preparation based on 1-pot (e.g., single vessel) reactions

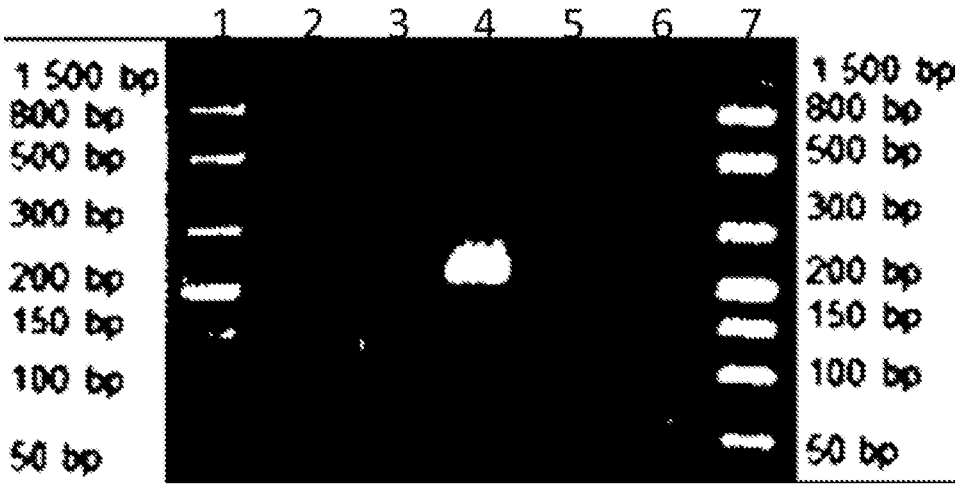


FIGURE 12C

Gel showing amplicon

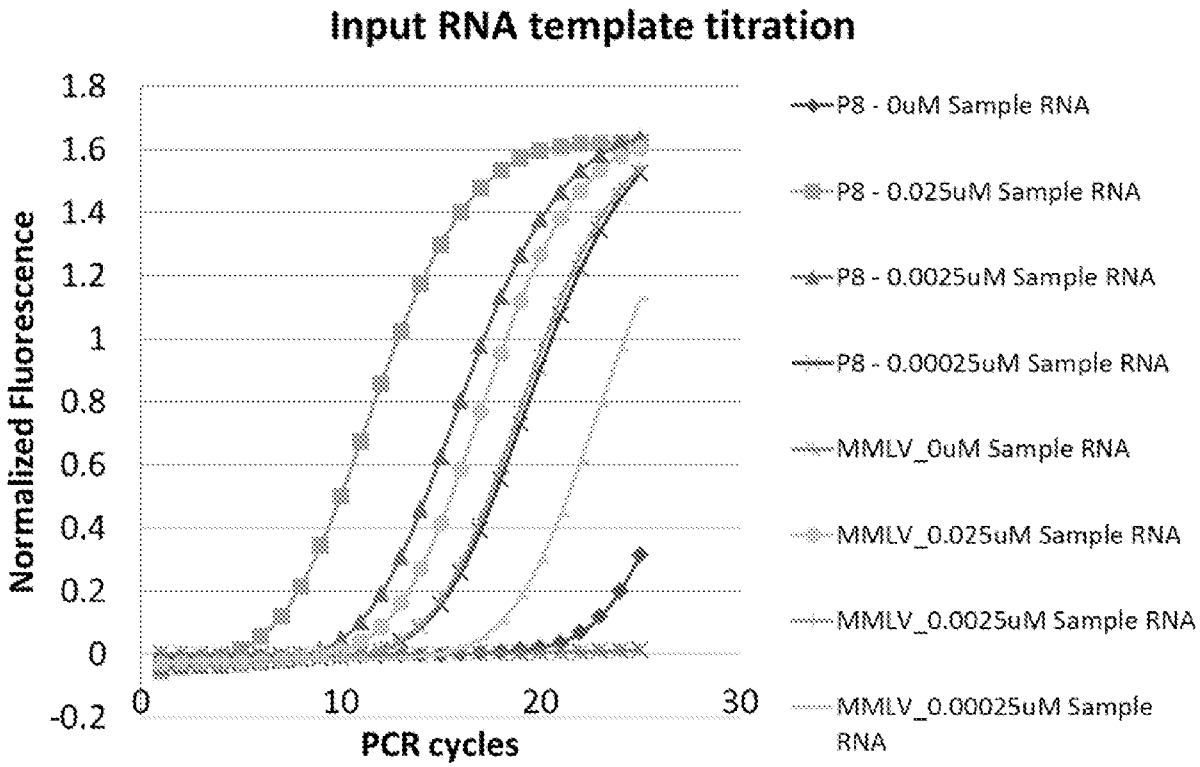


FIGURE 13

1-pot (e.g., single vessel) RNA library prep using different template amounts

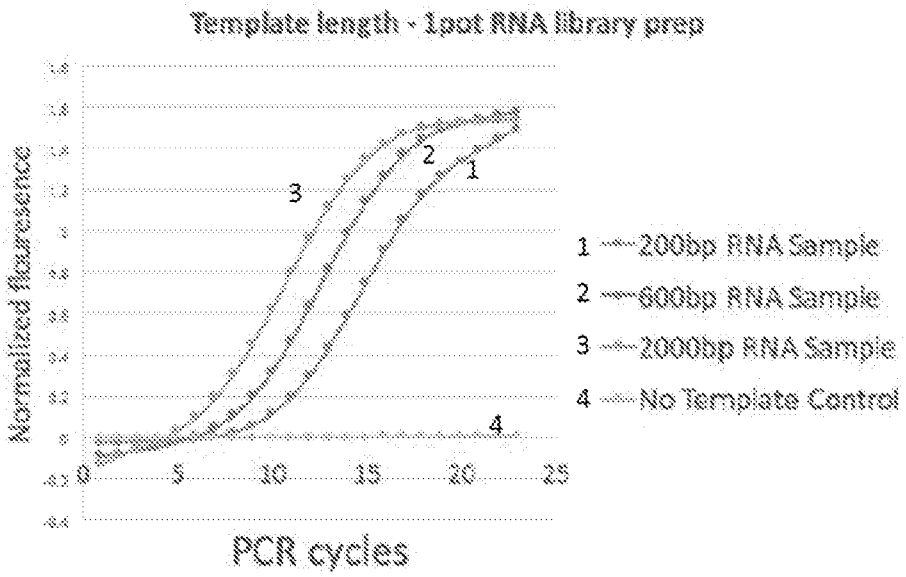


FIGURE 14

1-pot (e.g., single vessel) RNA library prep using different template lengths

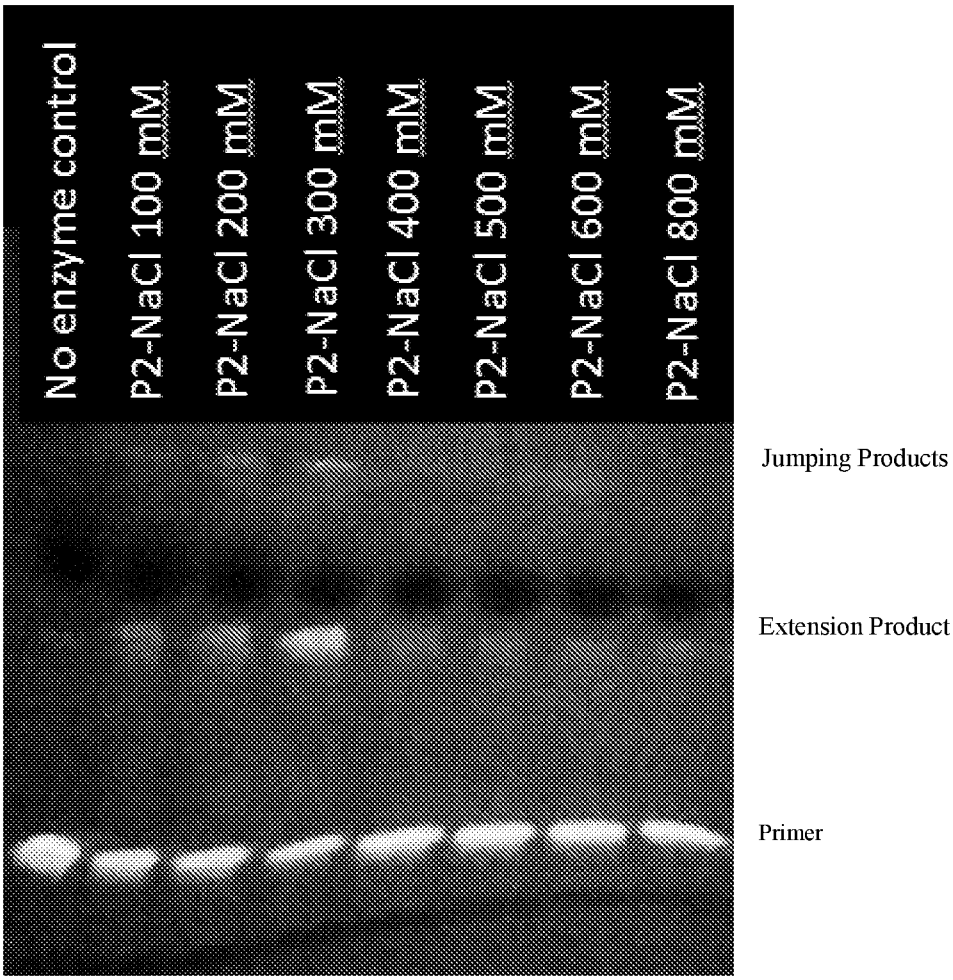


FIGURE 15

Enzyme activity and template jumping is dependent on NaCl concentration

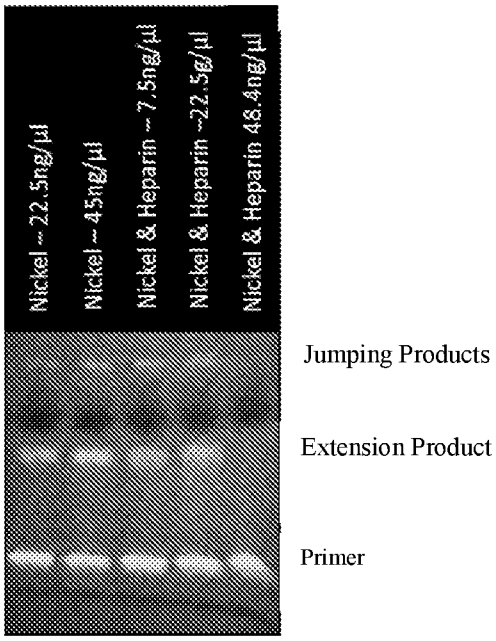


FIGURE 16A

Enzyme activity based on nickel and/or heparin affinity purification

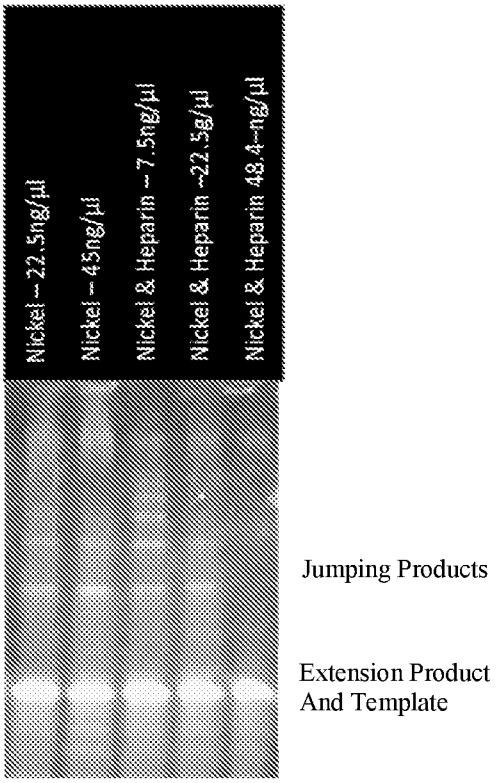


FIGURE 16B

Template jumping properties based on nickel and/or heparin affinity purification

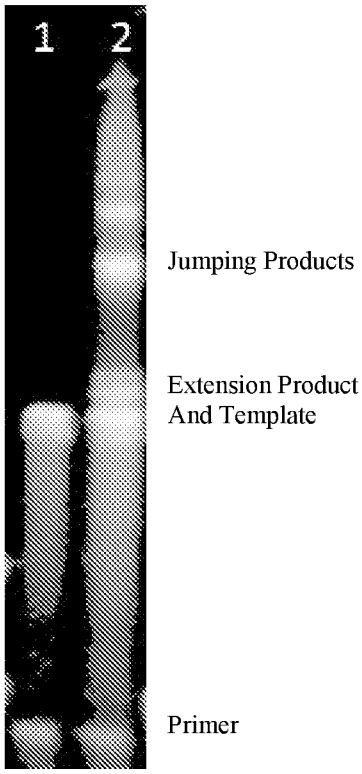


FIGURE 17

R2 enzyme activity and template jumping in the presence of DNA template (lane 1: no enzyme control; lane 2: 0.023 μg/μl enzyme in the presence of DNA template)

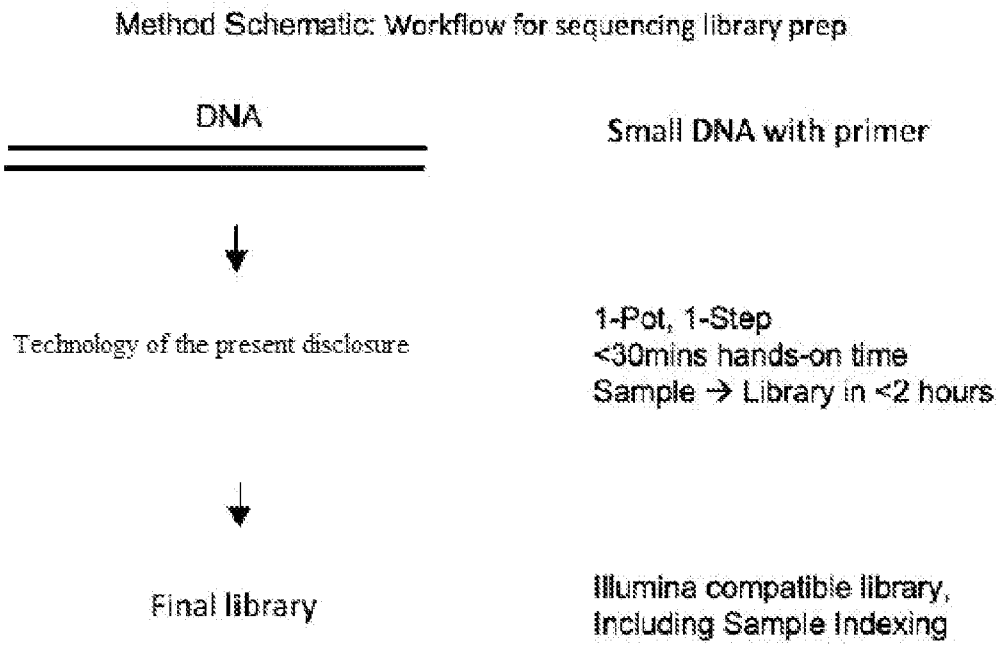


FIGURE 18A

Workflow of a method of the present disclosure for sequencing a library preparation from liquid biopsy

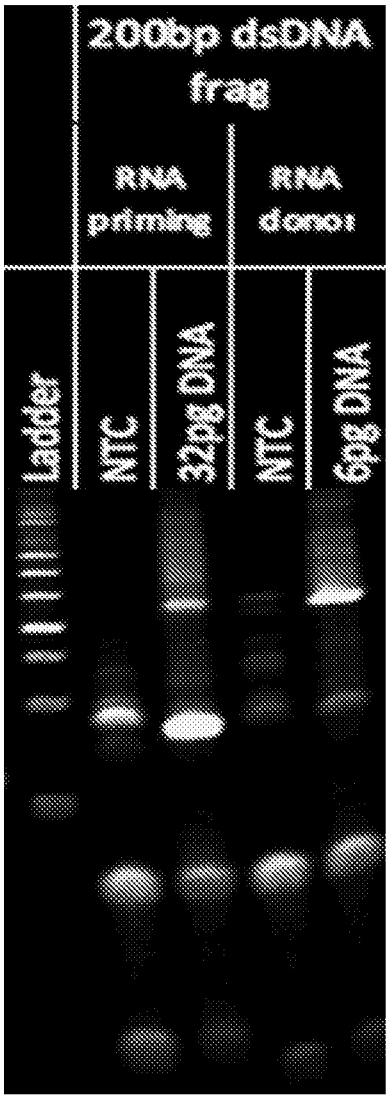


FIGURE 18B

Sample DNA fragment was captured by an R2 enzyme with both an RNA priming approach and an RNA donor approach

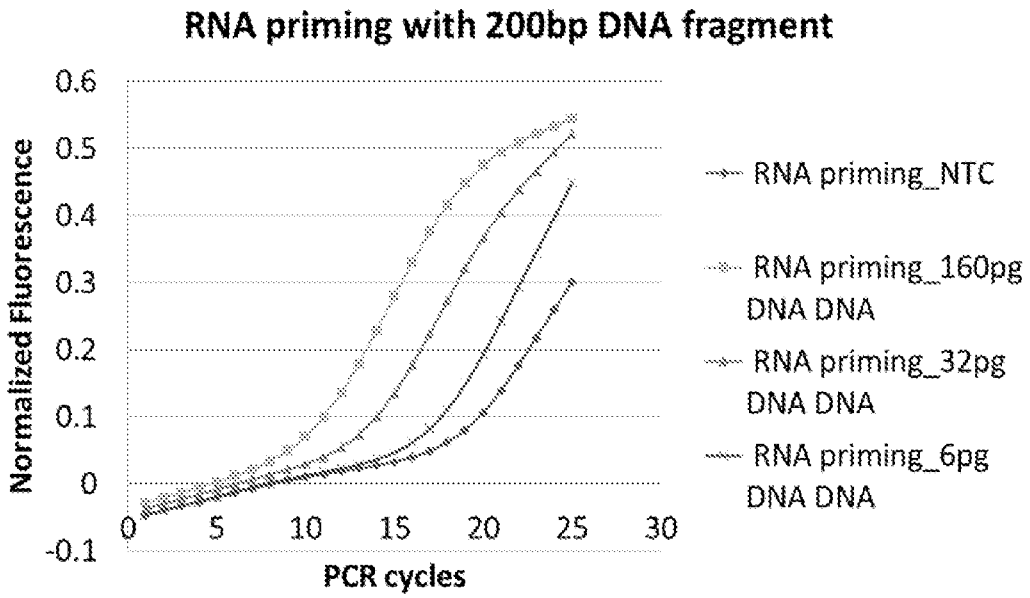


FIGURE 18C

Sample DNA fragment (at various concentrations) was captured by an R2 enzyme using an RNA priming approach

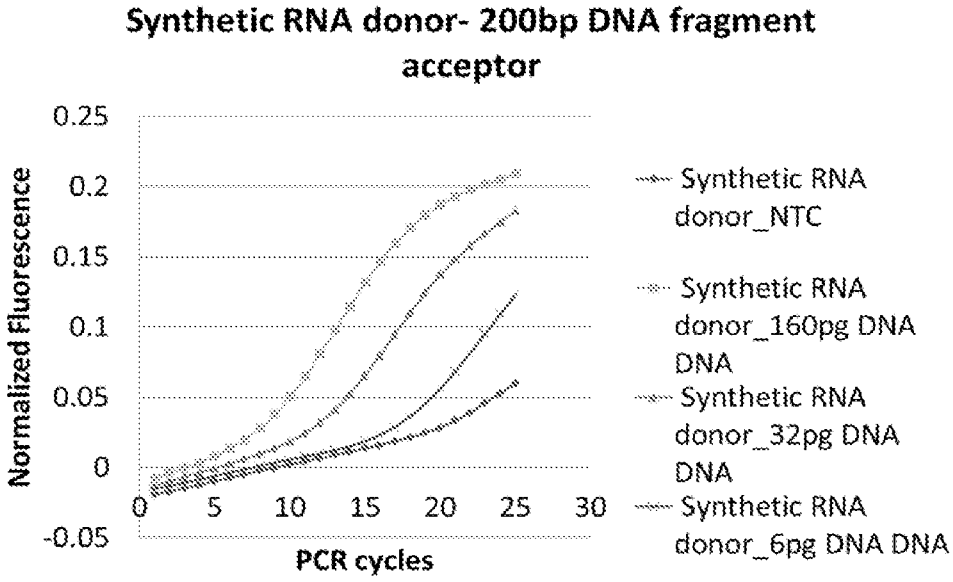


FIGURE 18D

Sample DNA fragment (at various concentrations) was captured by an R2 enzyme using an RNA donor approach

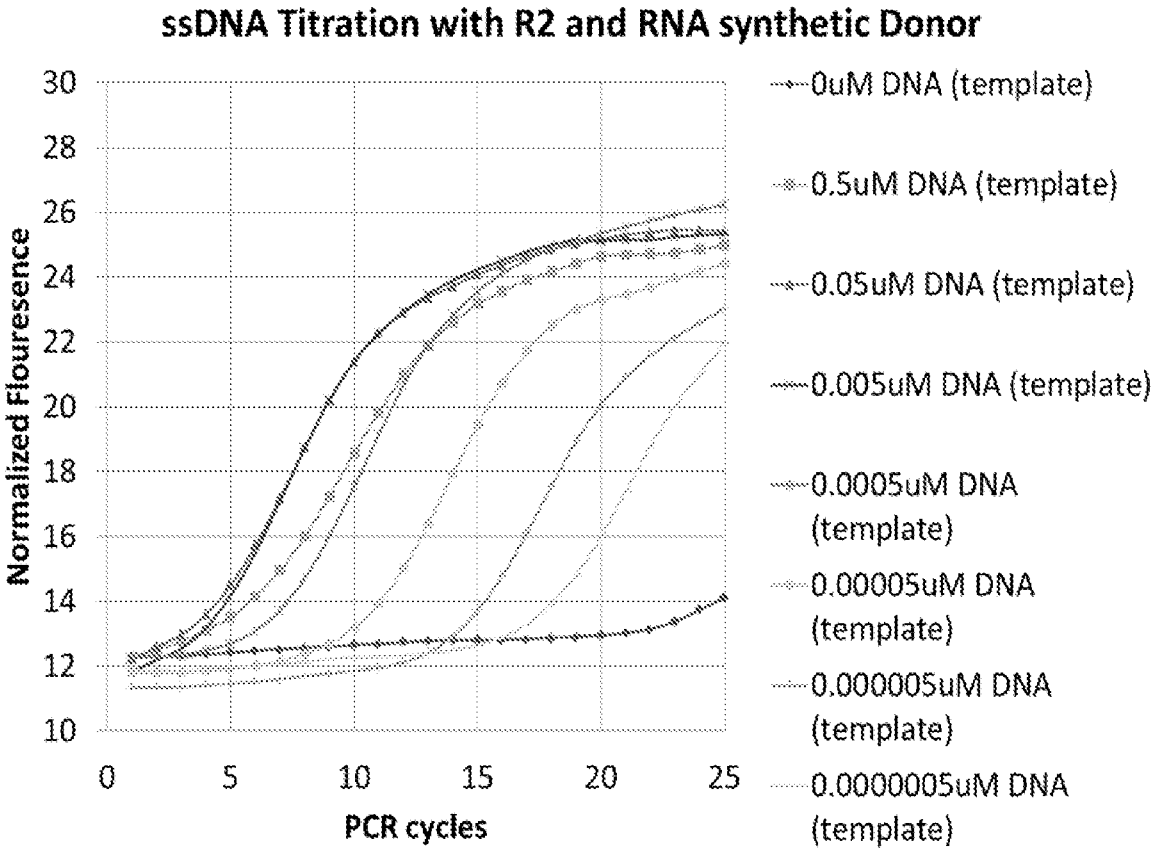


FIGURE 19

Sample DNA fragment was captured using an RNA donor approach at a concentration as low as 500 femtomolar

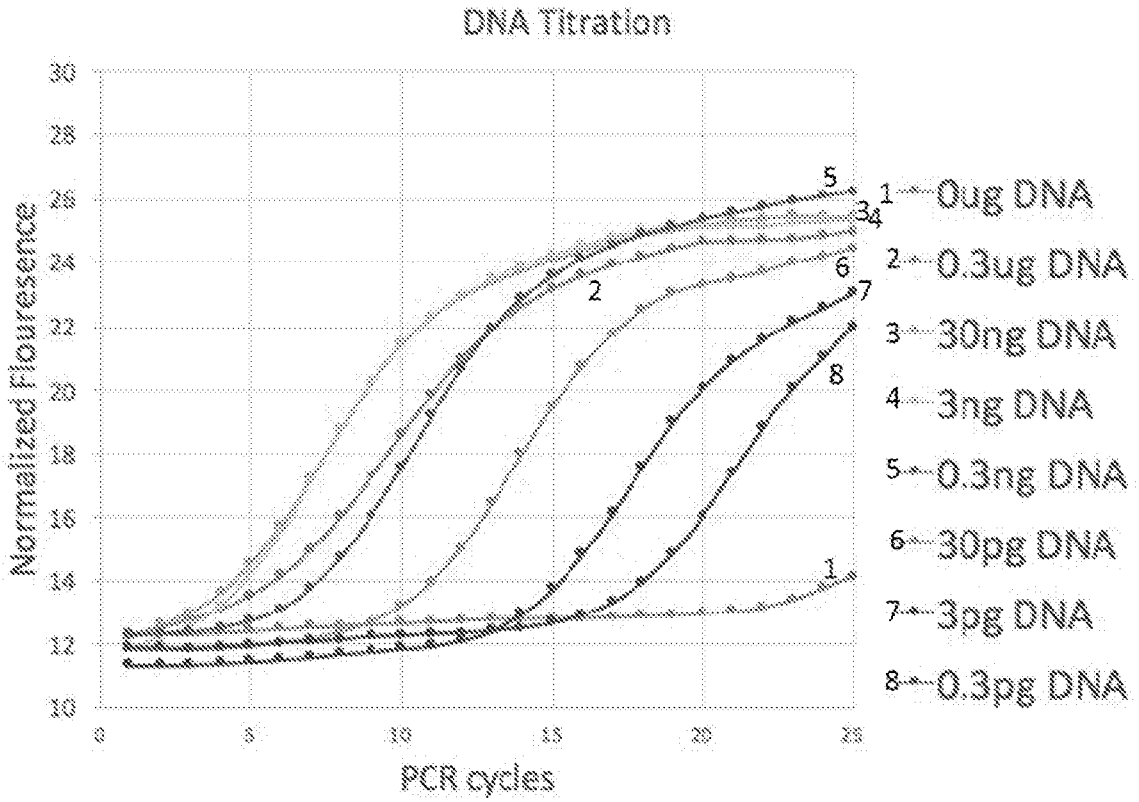


FIGURE 20

Sensitivity driver for liquid biopsy application. Data shows sensitivity up to 0.3 pg of DNA.

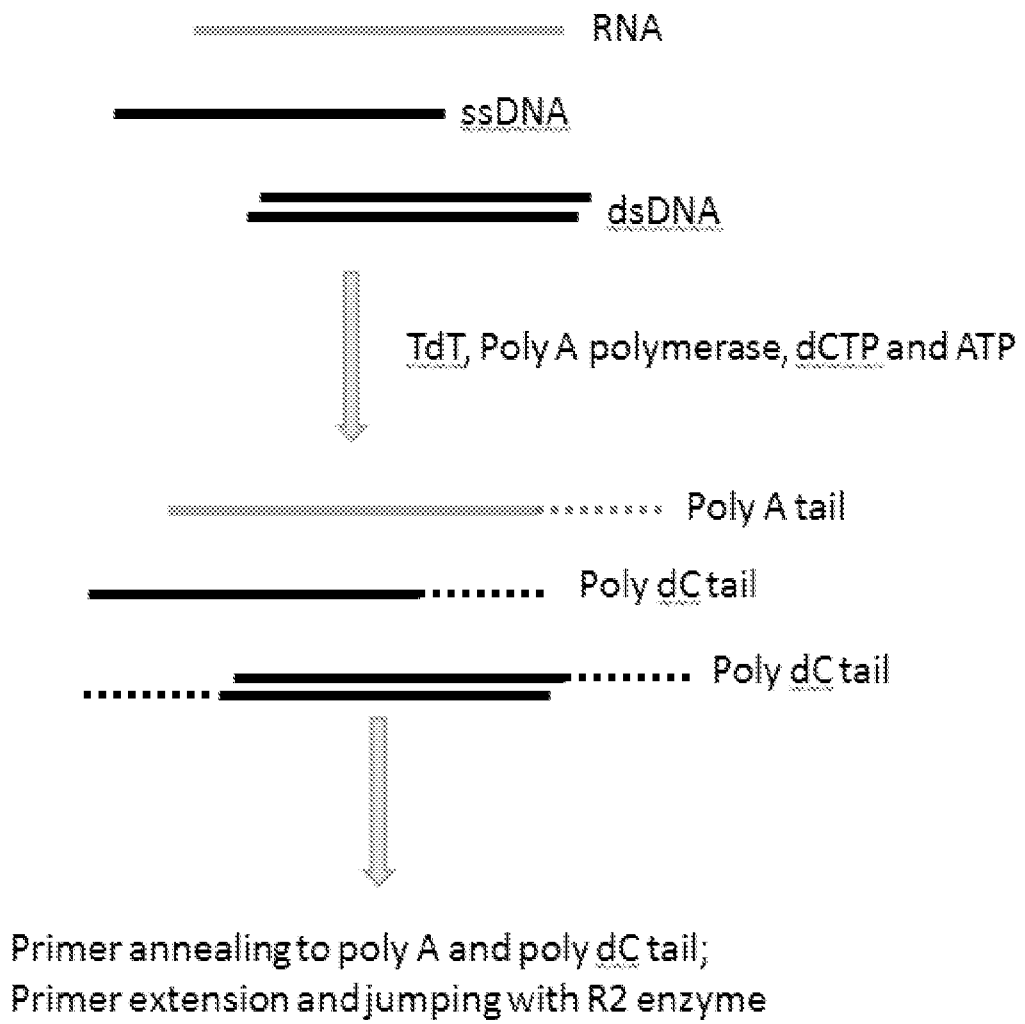


FIGURE 21

Schematic method for increasing the detection sensitivity of low frequency mutations by using cell-free nucleic acids comprising cell-free DNA and RNA.

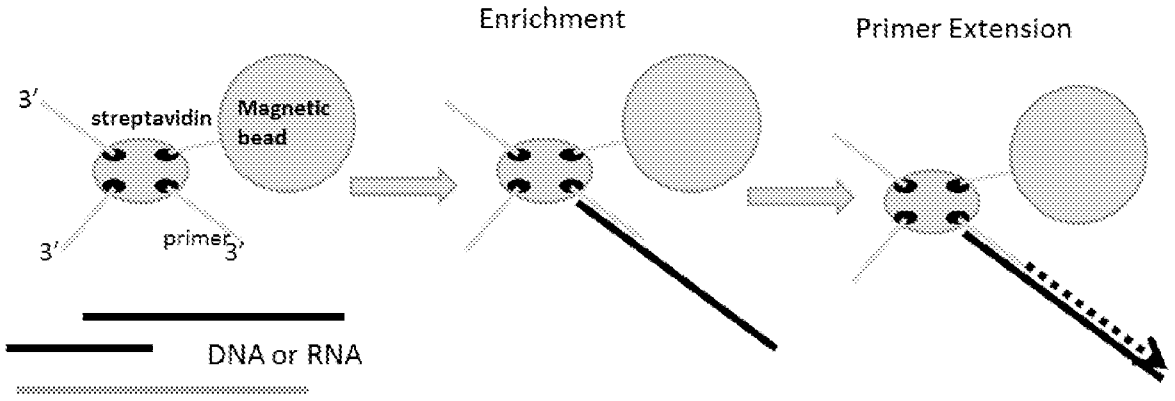


FIGURE 22A

Improvement in priming, specific template capture and template jumping efficiency by using streptavidin-immobilized oligonucleotides capable of binding to specific DNA and/or RNA template(s).

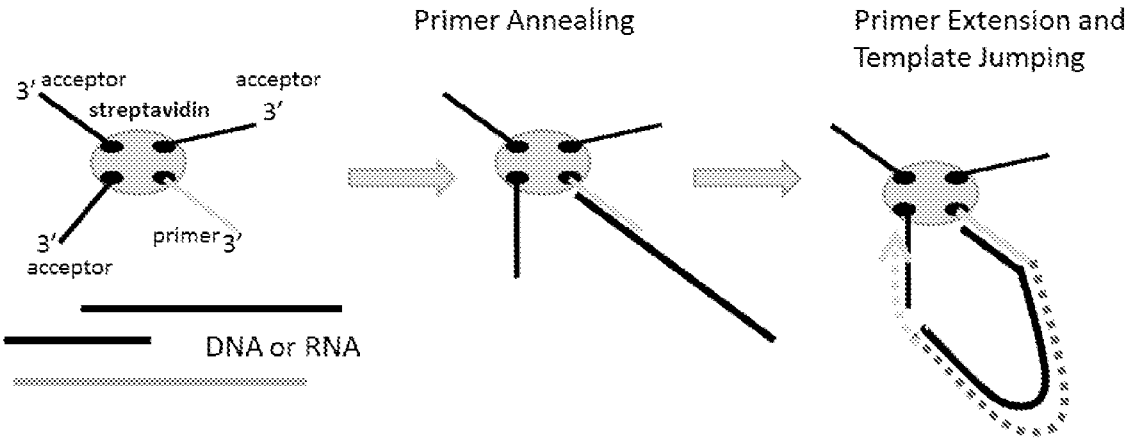


FIGURE 22B

A complex comprising streptavidin bound to both an oligonucleotide primer and an oligonucleotide acceptor is capable of binding to specific DNA and/or RNA template(s), allowing for primer extension and template jumping.

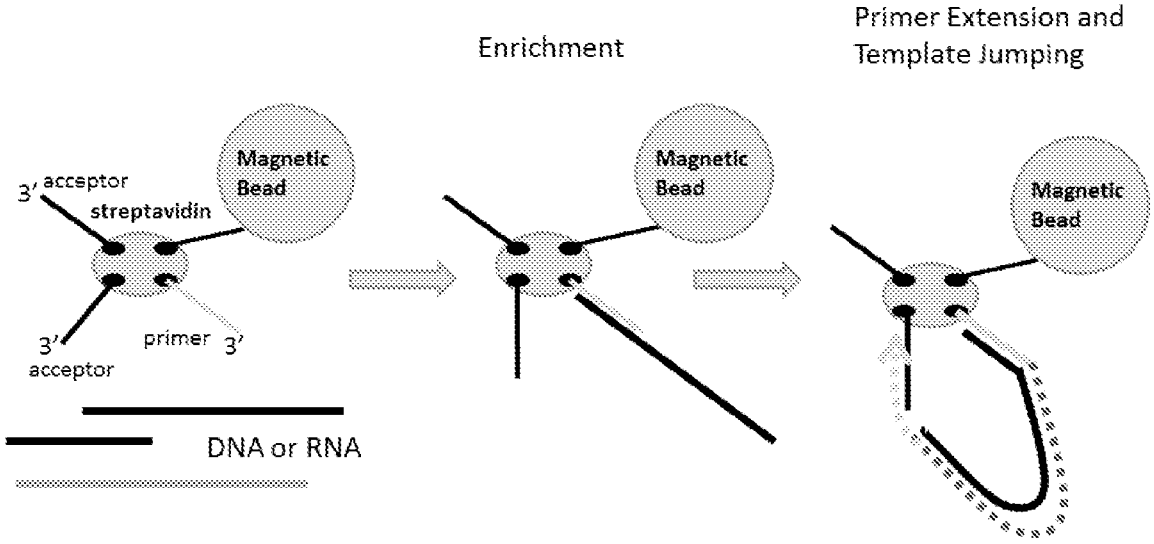


FIGURE 22C

Streptavidin bound to a magnetic bead allows for enrichment of DNA and/or RNA template(s).

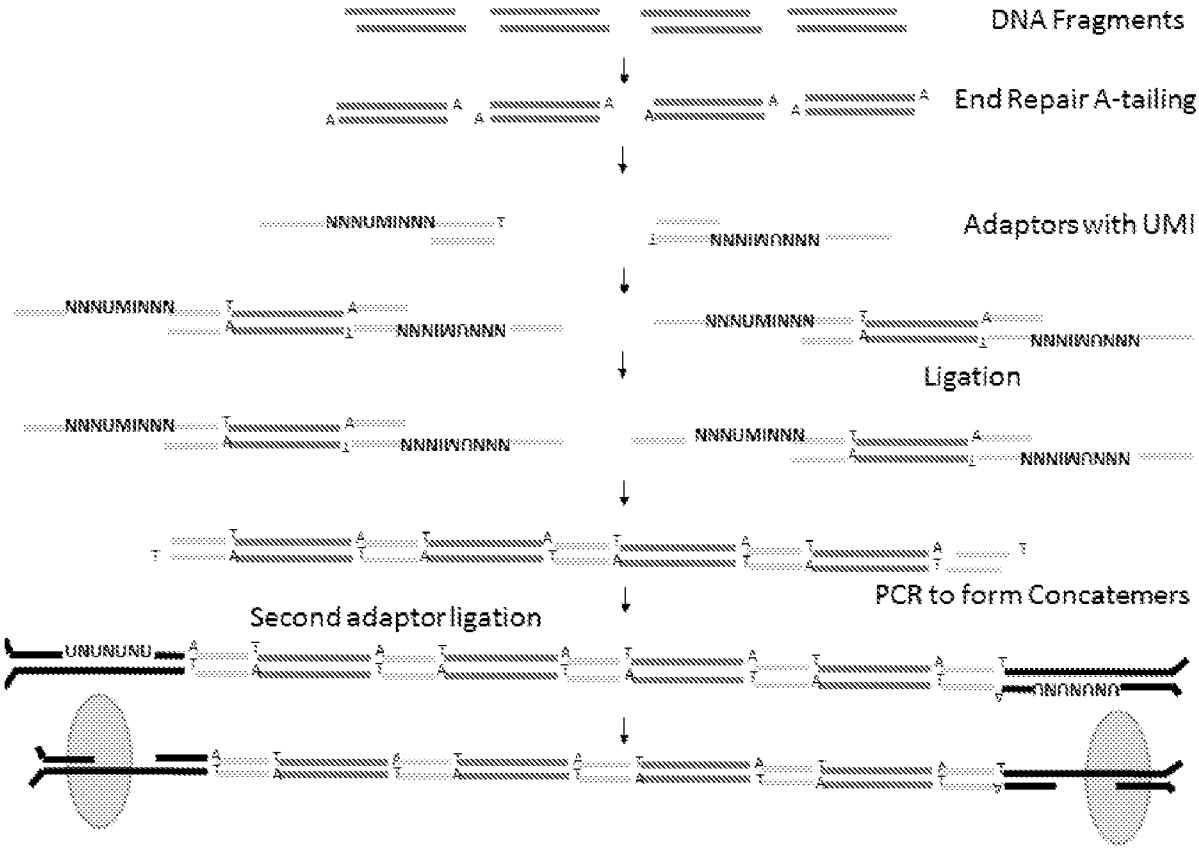


FIGURE 23A

Template Concatemerization

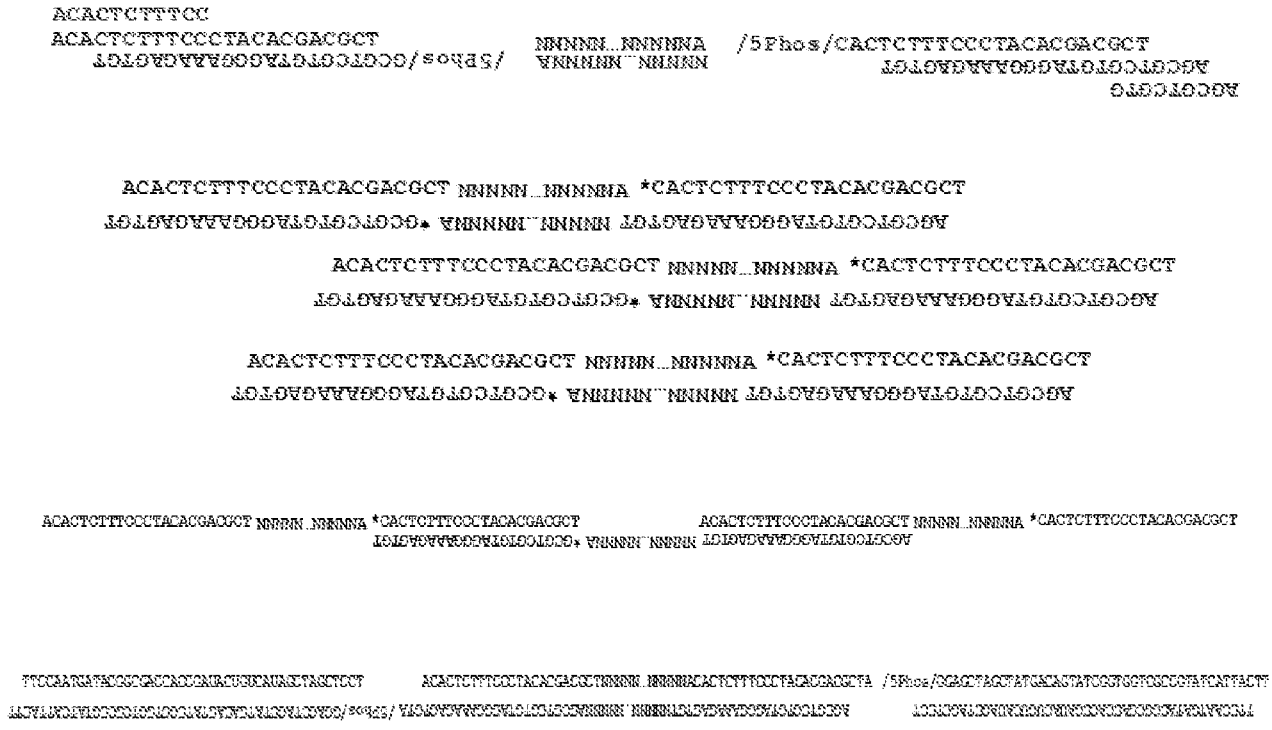


FIGURE 23B

Product of a concatemerization that includes specific adaptors on both ends

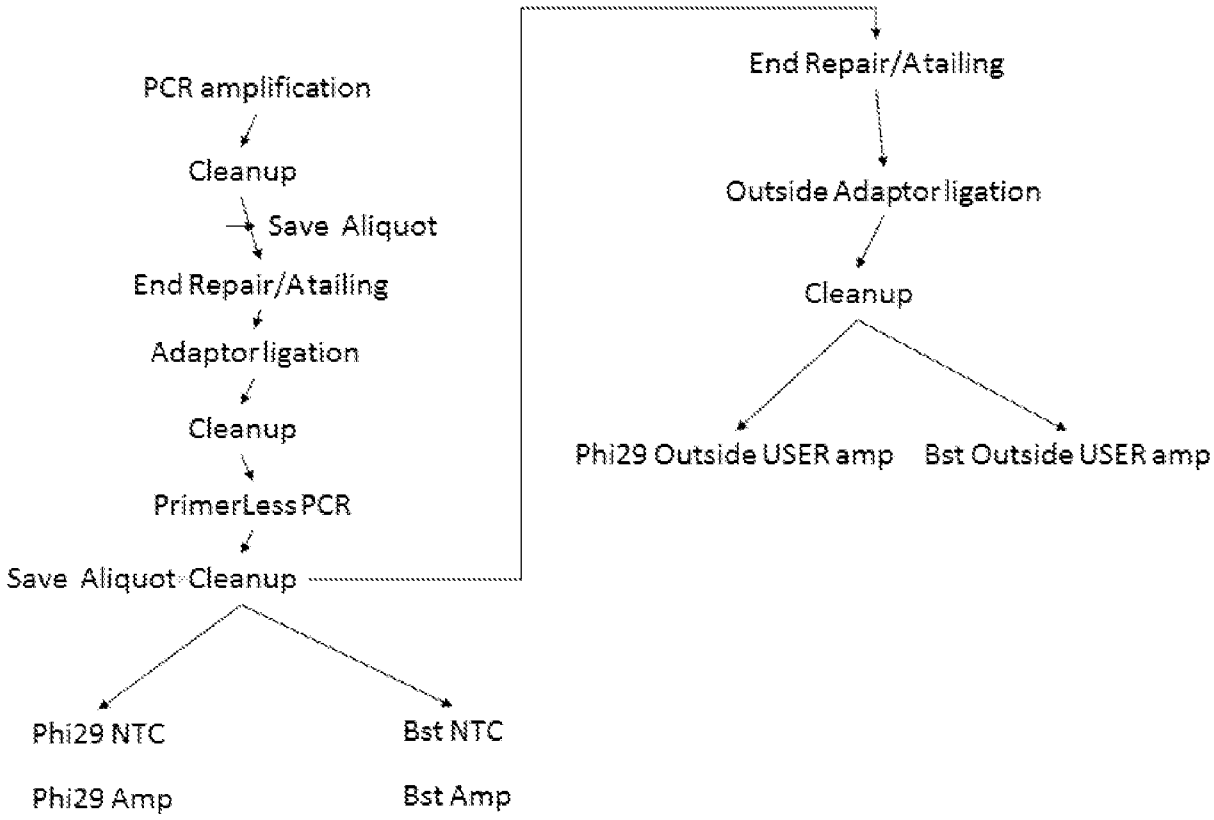


FIGURE 24A

Workflow of template concatemerization

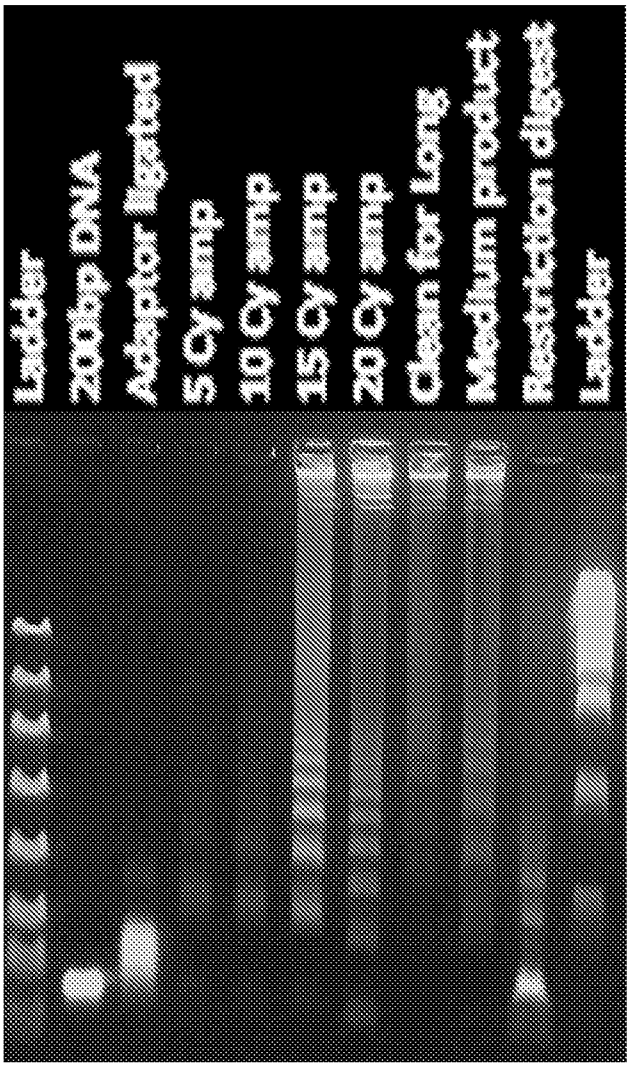


FIGURE 24B

Gel showing the concatemerization of a 200bp DNA fragment

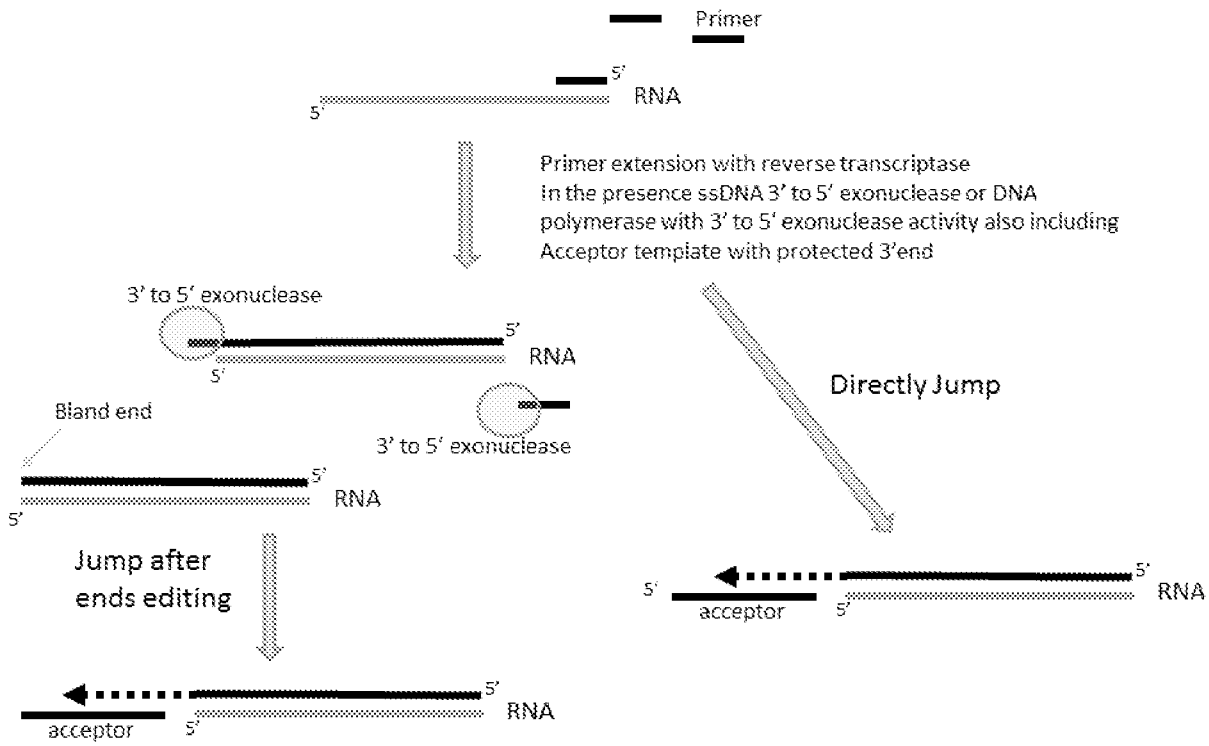


FIGURE 25

Schematic representation of primer extension using a reverse transcriptase and a companion enzyme

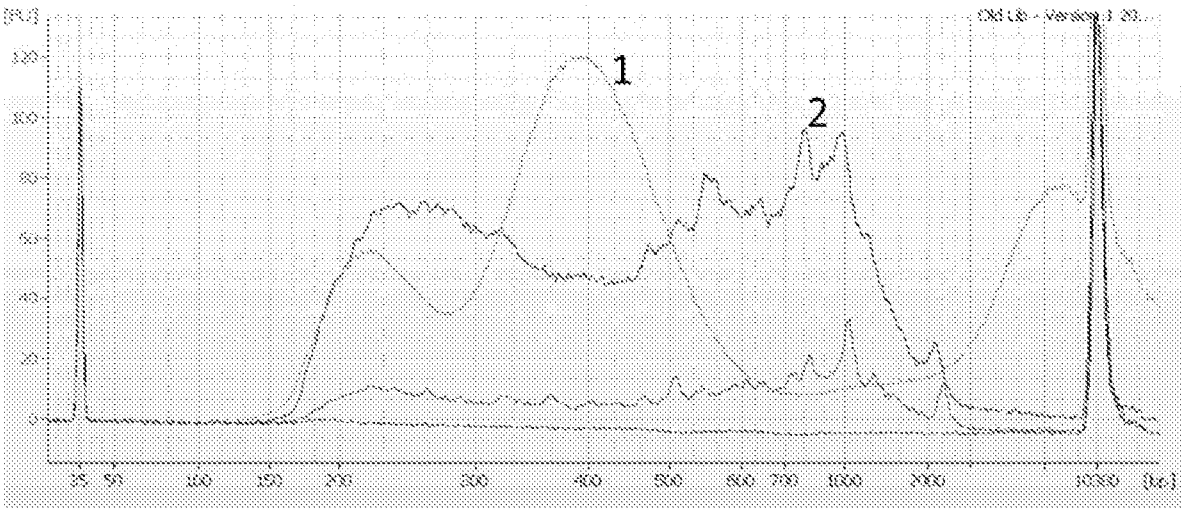


FIGURE 26

BioAnalyzer trace data of next-generation sequencing (NGS) libraries: (1) Fragmented RNA seq library from plasma; (2) not fragmented RNA seq library from plasma

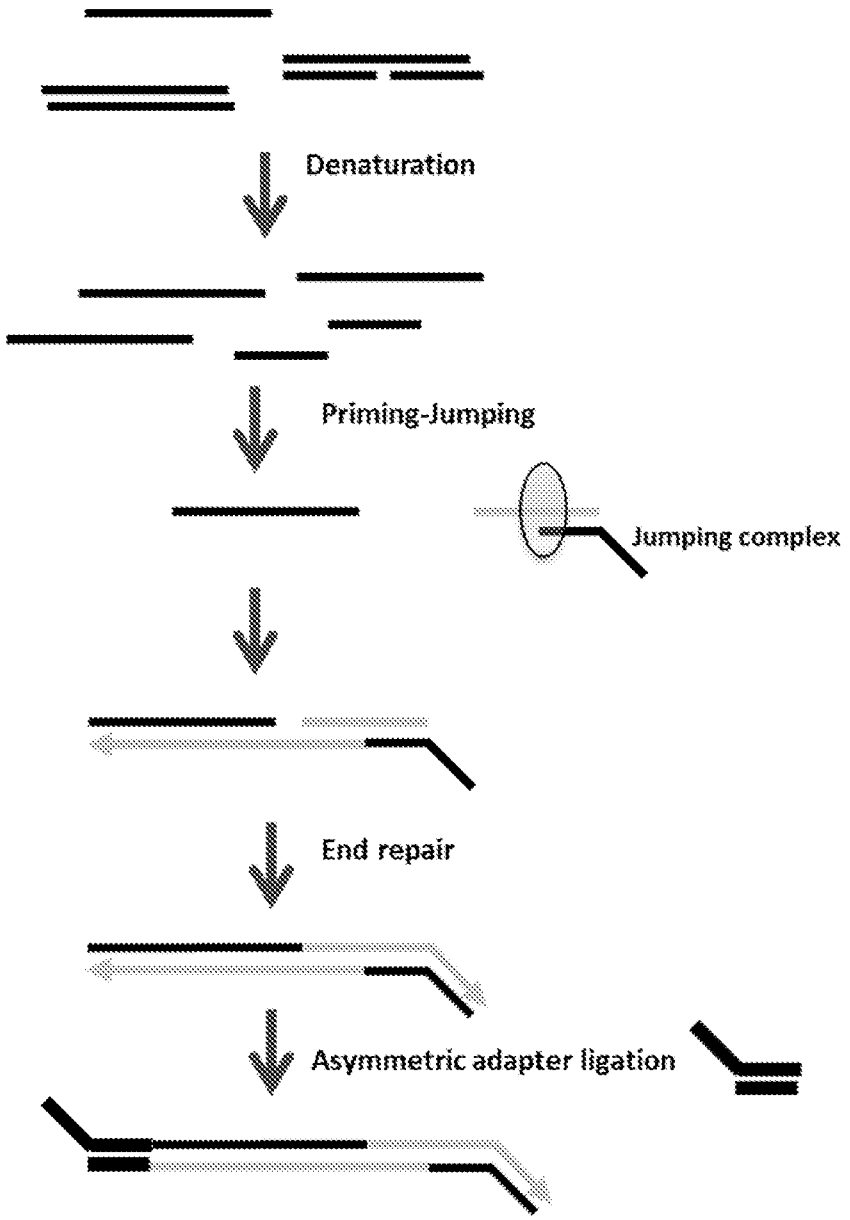


FIGURE 27

Method of generating cfDNA library

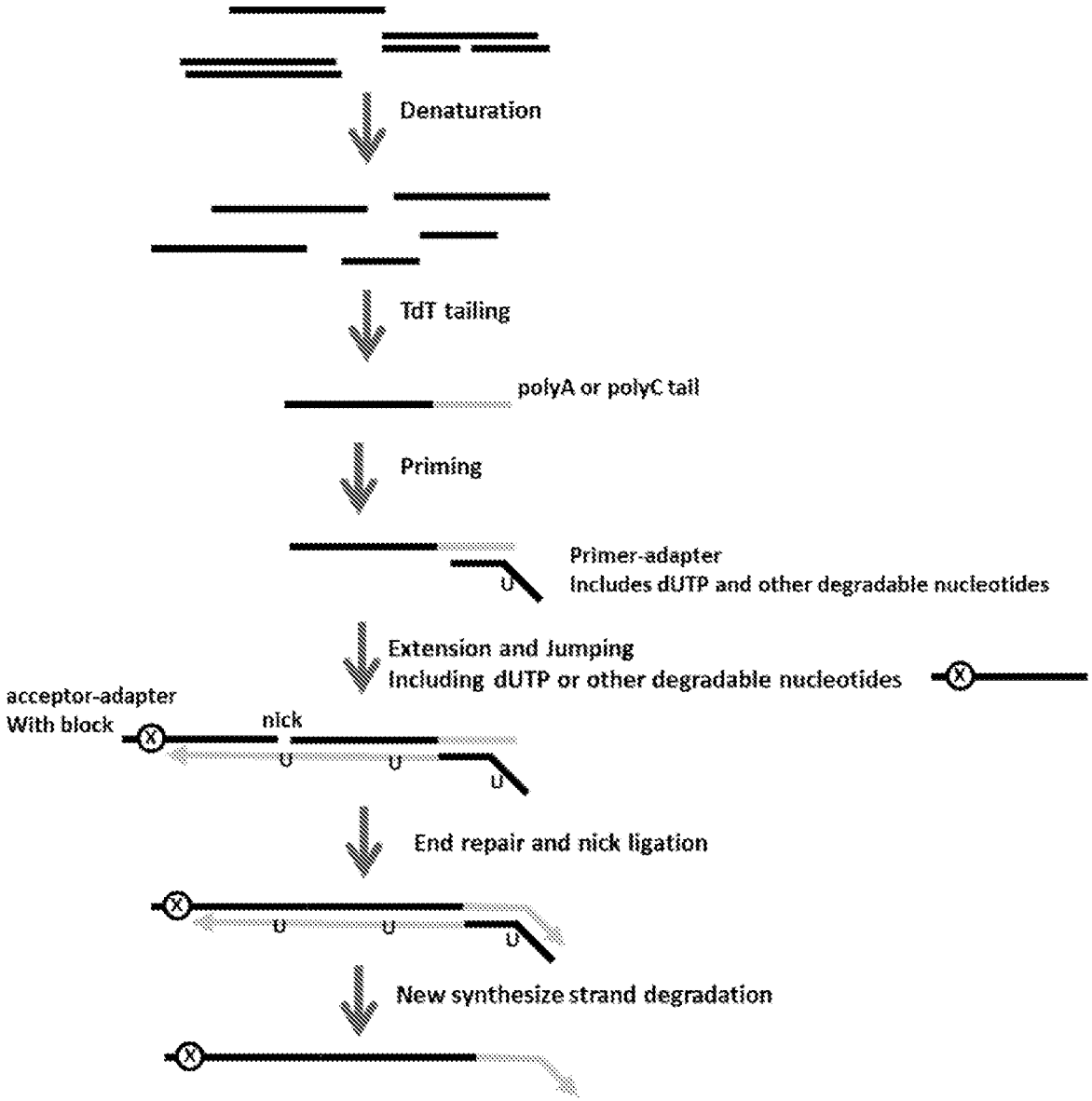


FIGURE 28

Method of generating cfDNA library

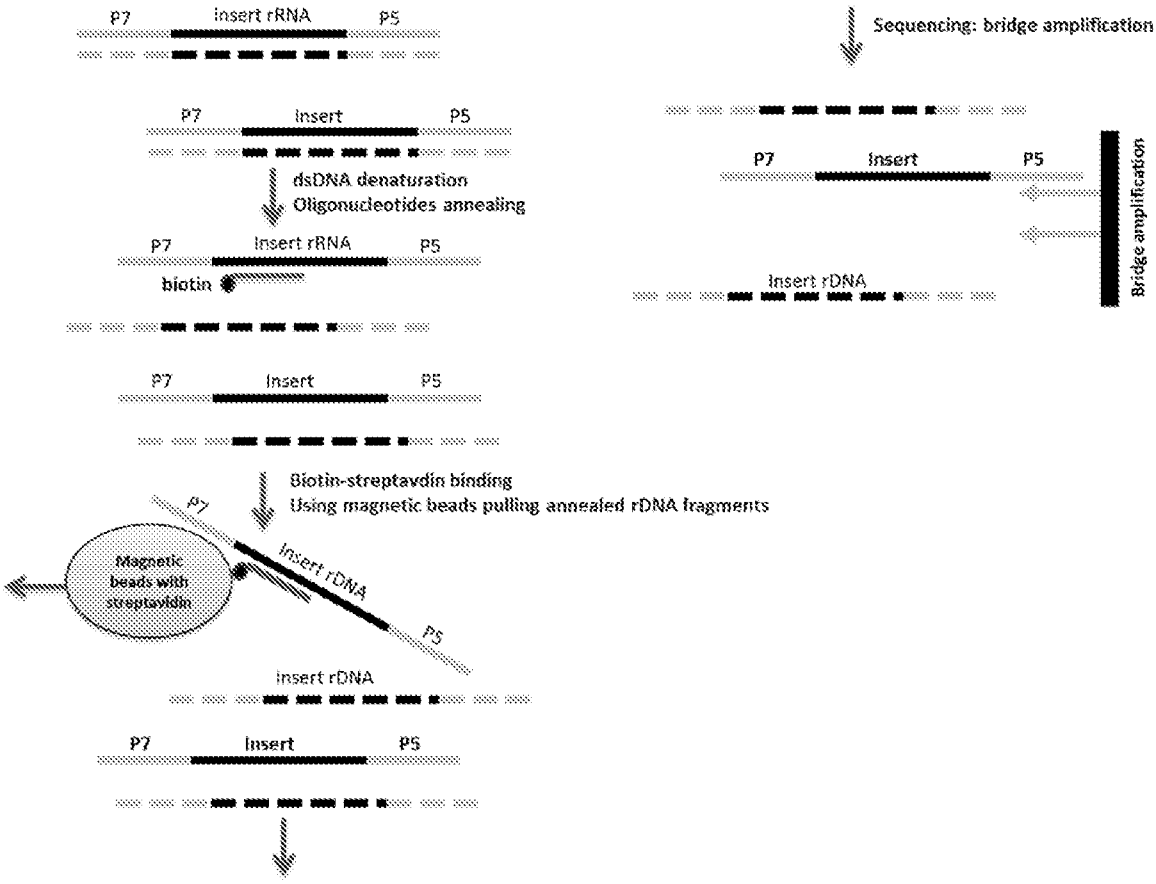


FIGURE 29

Library preparation: pulling ribosomal RNA and/or transfer RNA and/or PCR products using complementary oligonucleotide attached to magnetic beads or solid support to maximize sequencing capacity

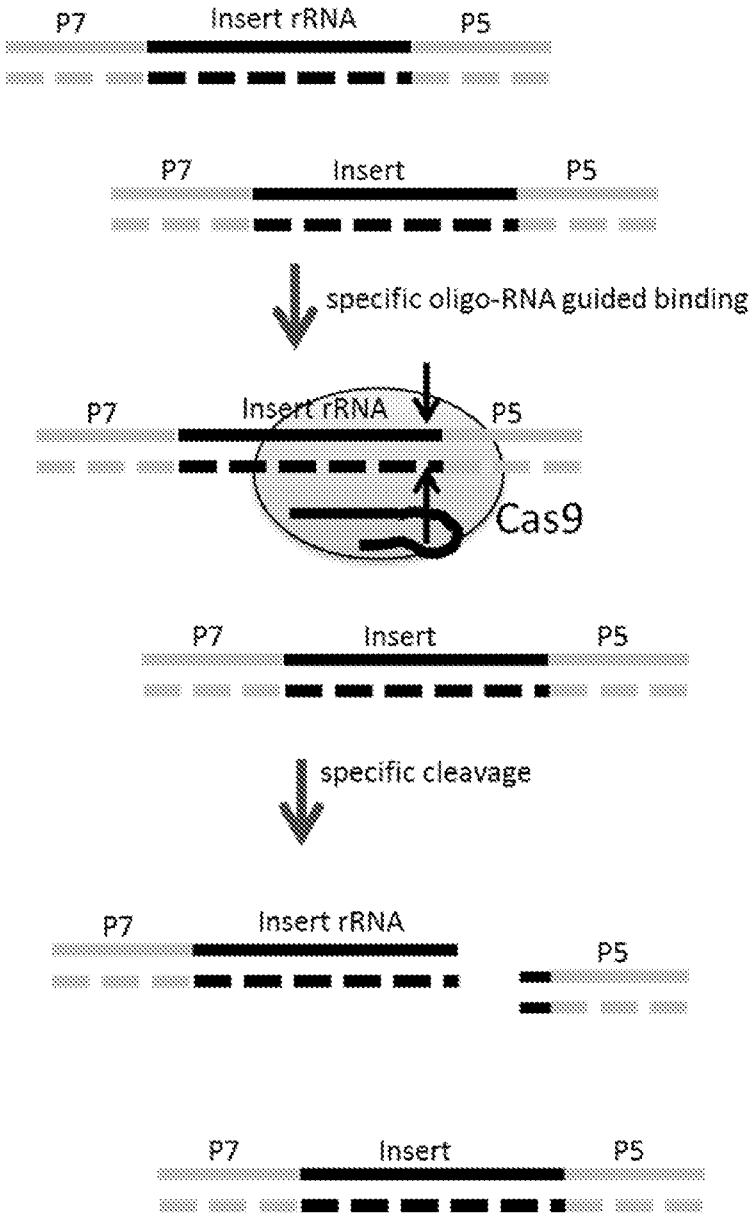


FIGURE 30

Library preparation: oligonucleotide-guided degradation of ribosomal RNA and/or transfer RNA and/or PCR products to maximize sequencing capacity

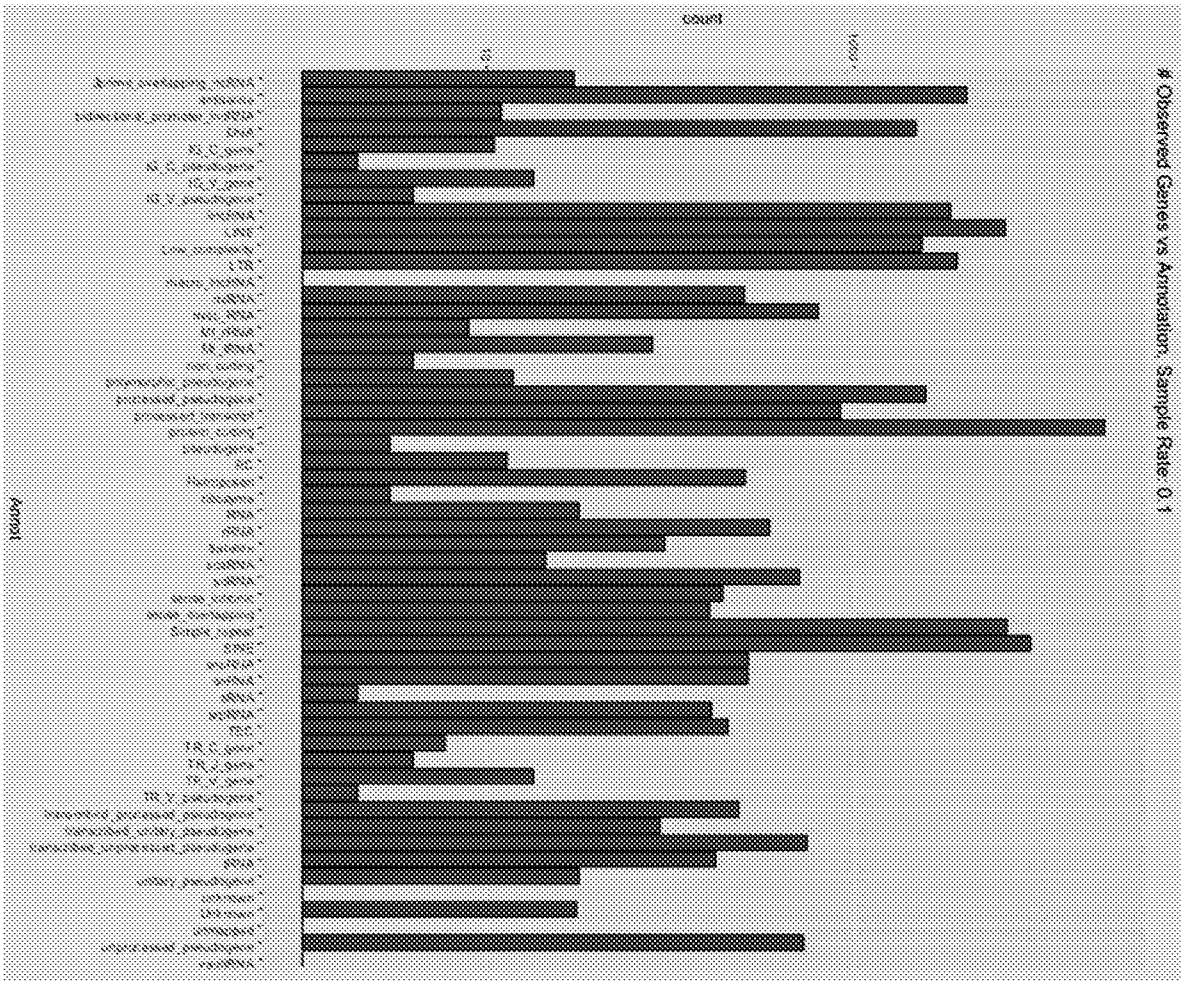


FIGURE 31

Technology capable of targeting all species of RNA simultaneously