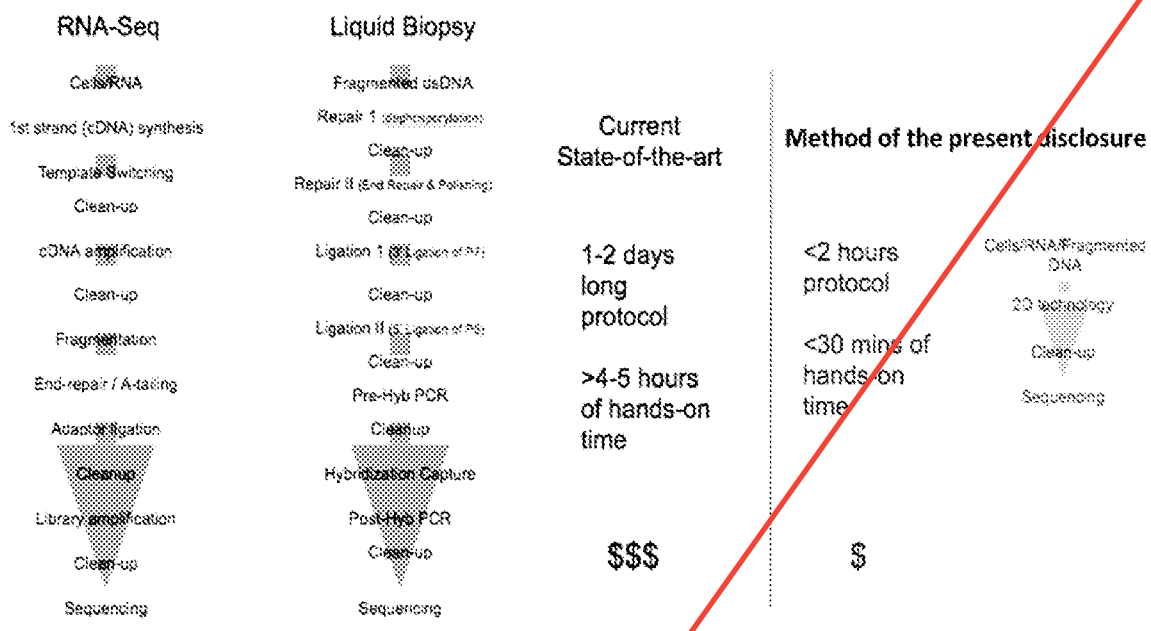


FIGURE 1

Workflow comparison

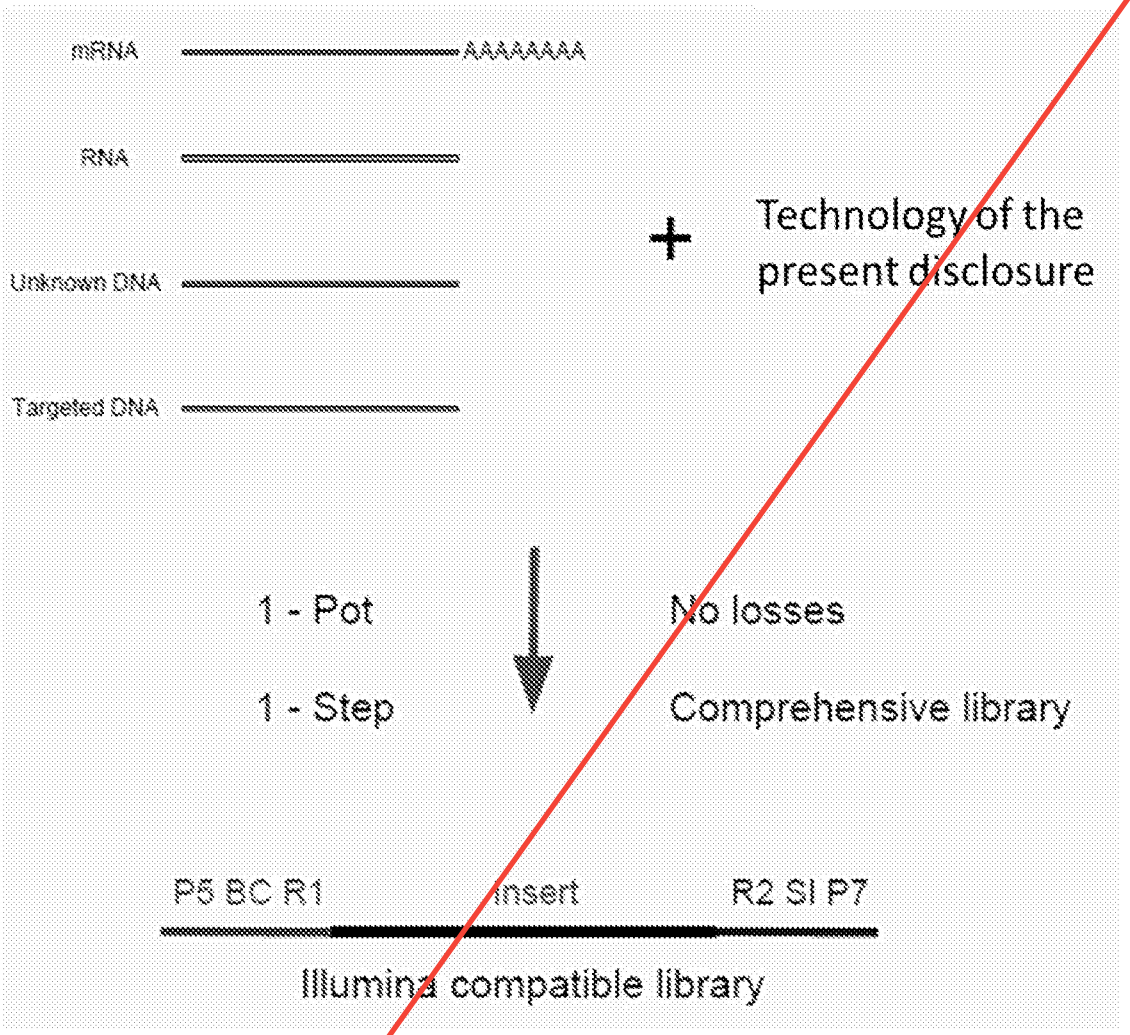


FIGURE 2

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

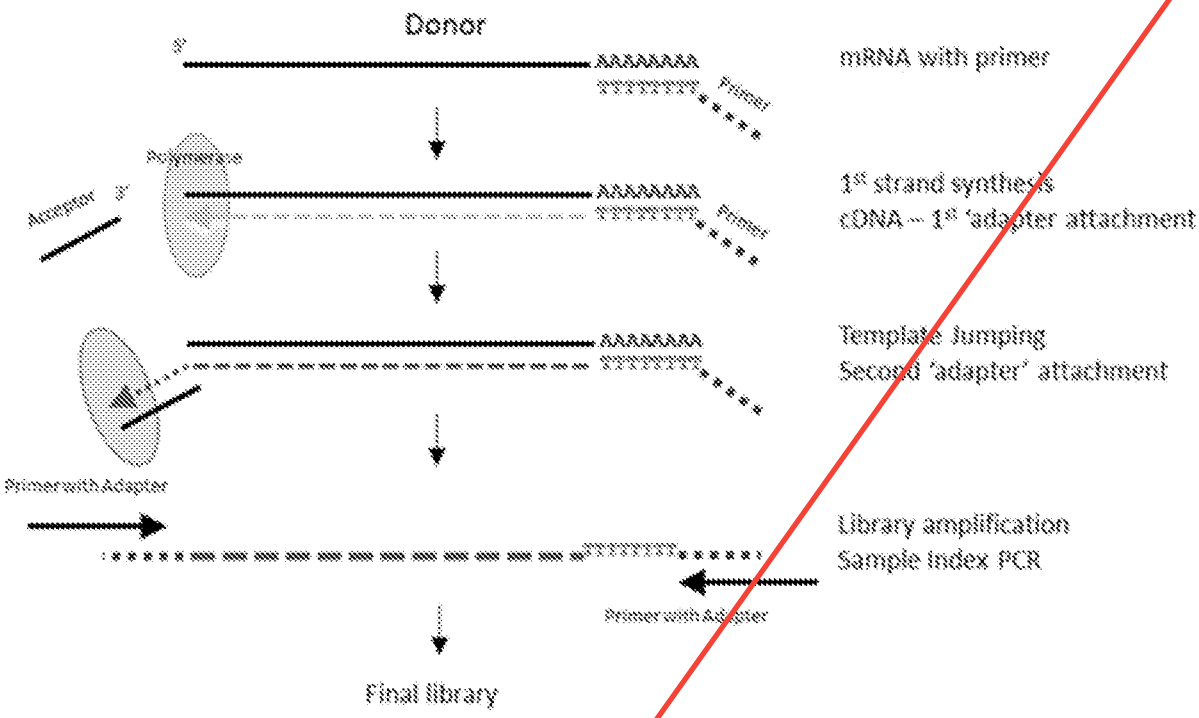


FIGURE 3

Example of a workflow for constructing a library

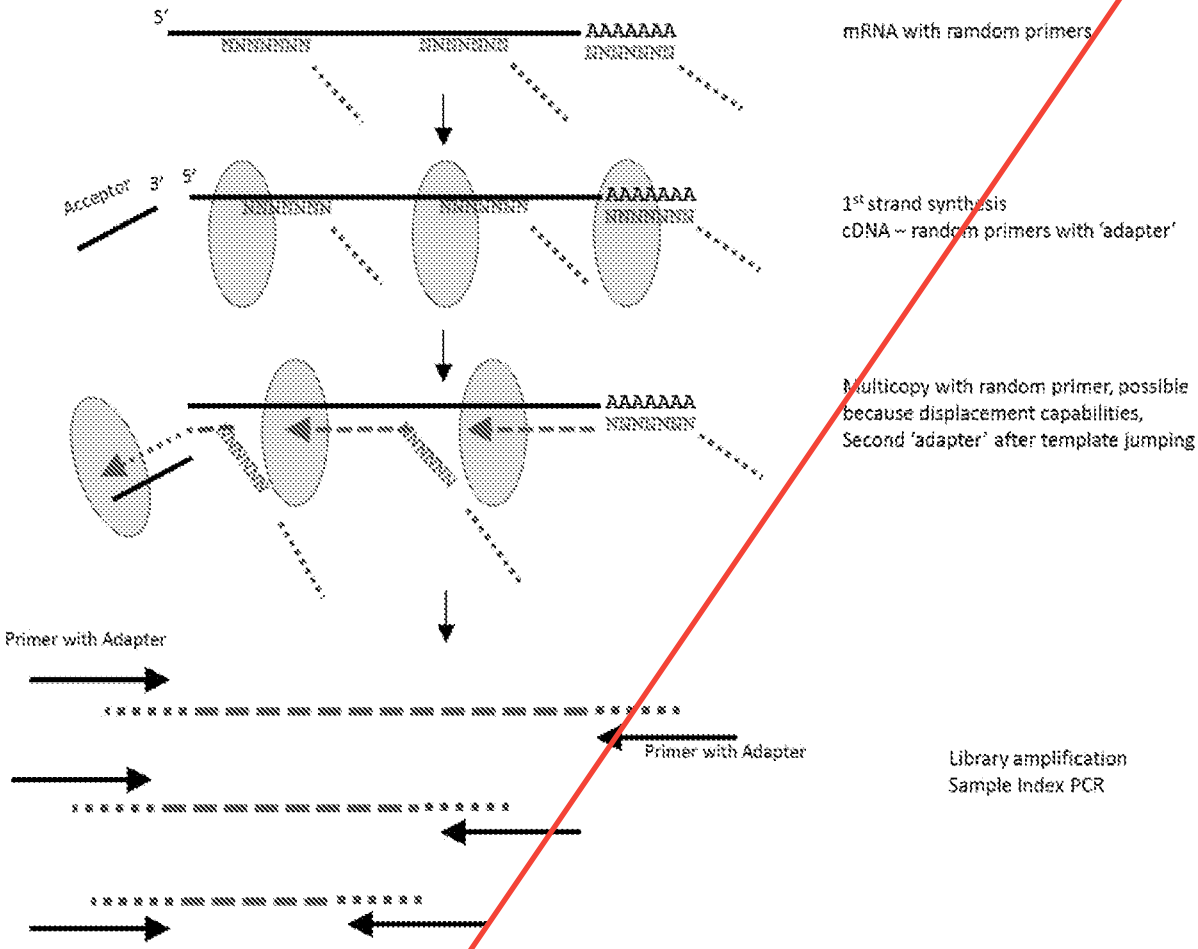


FIGURE 4

Example of a workflow using random primers for constructing a library

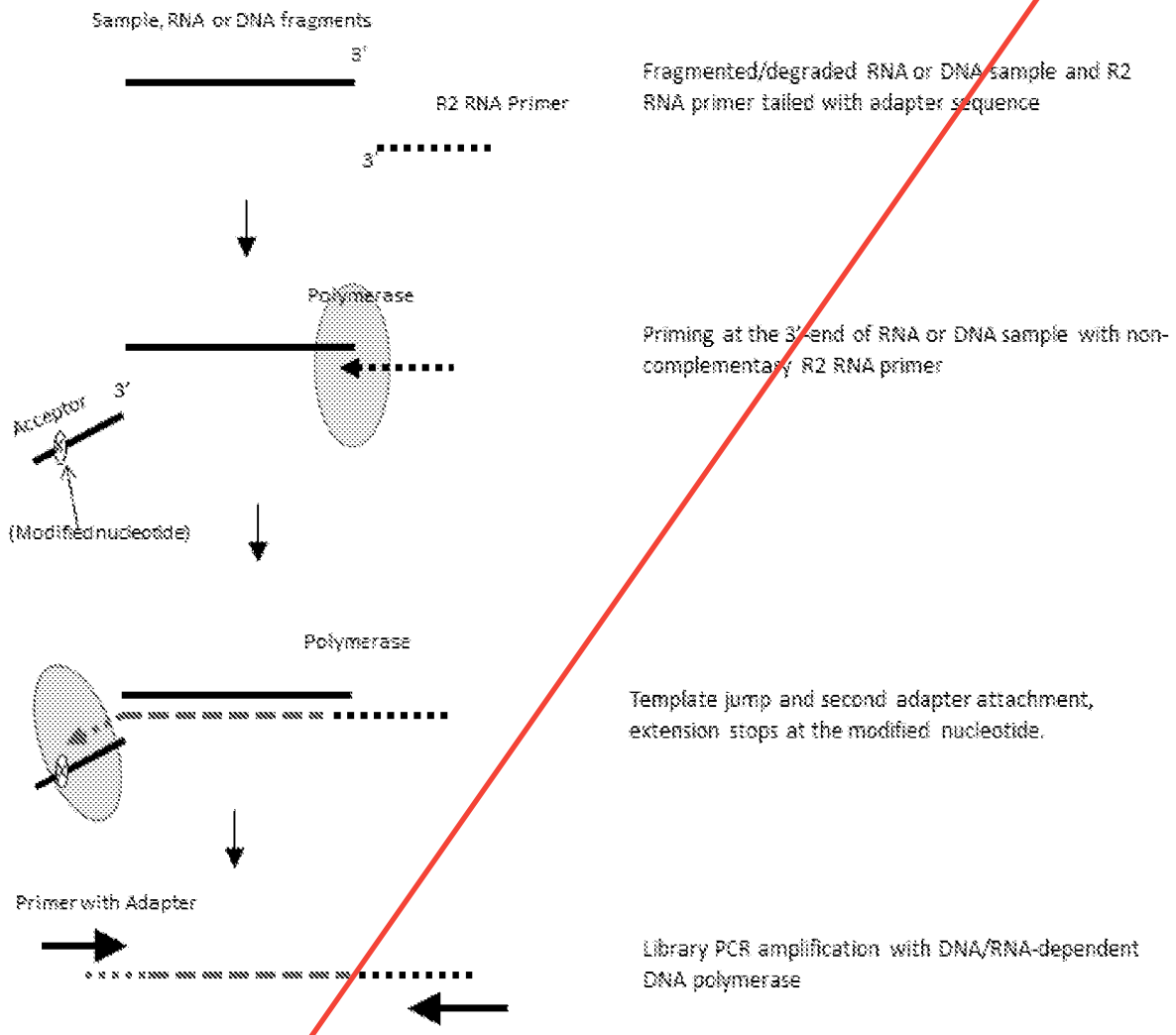


FIGURE 5A

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

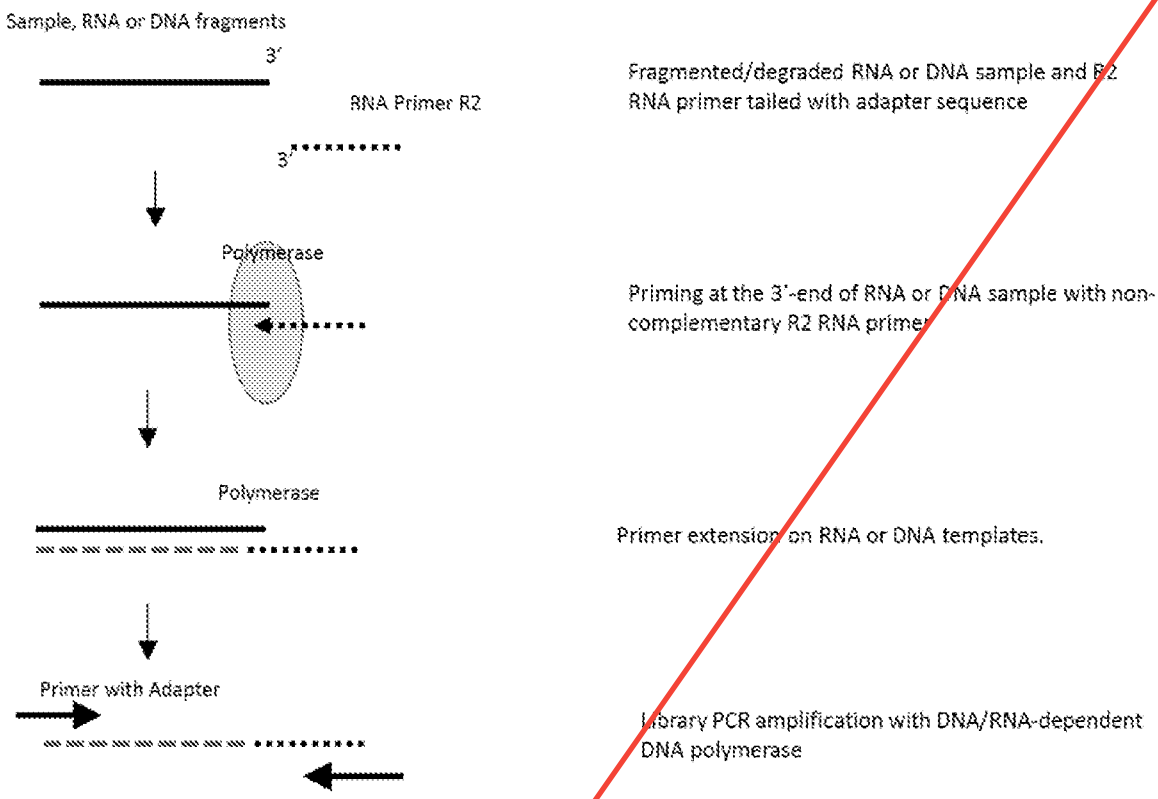


FIGURE 5B

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

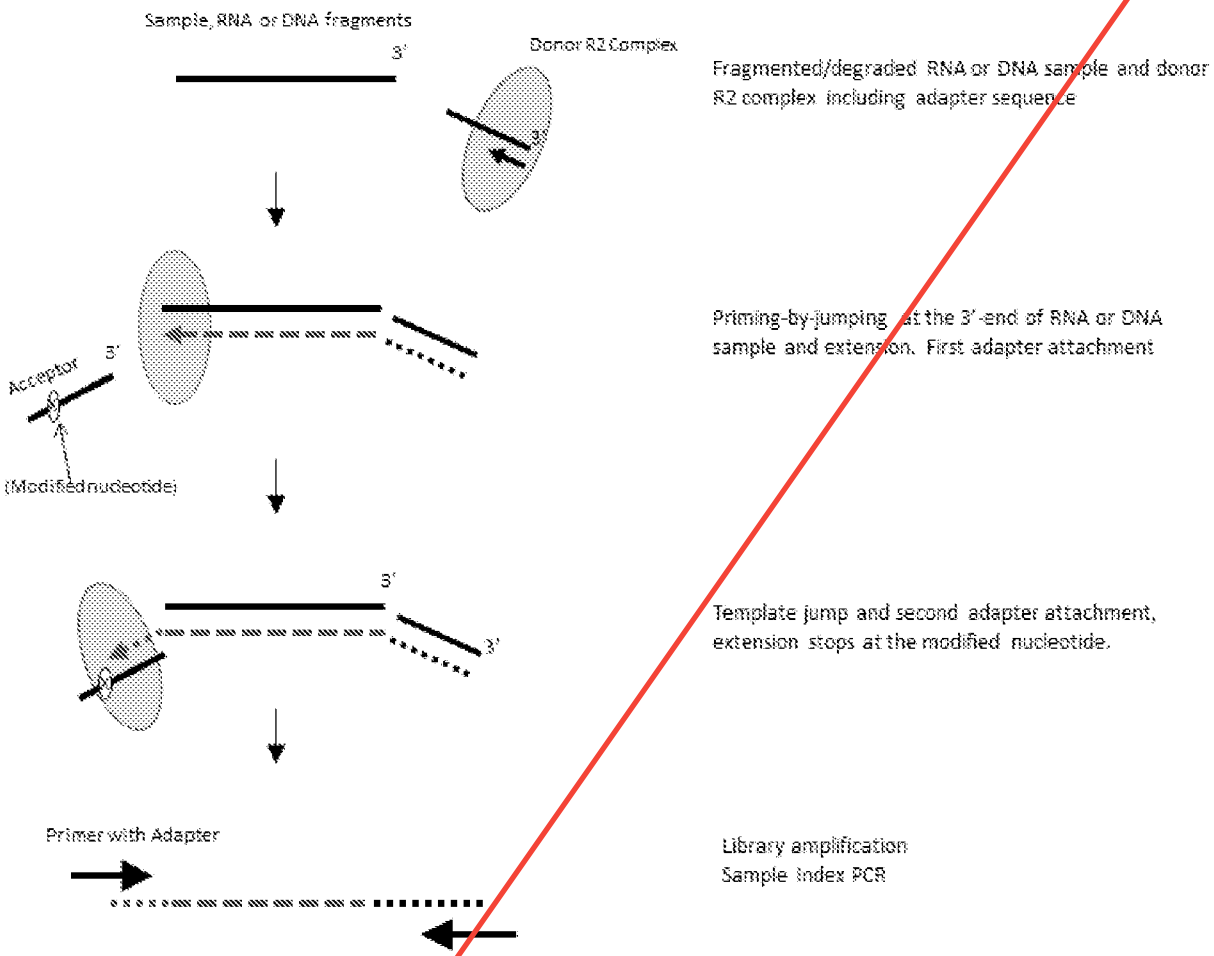


FIGURE 6A

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

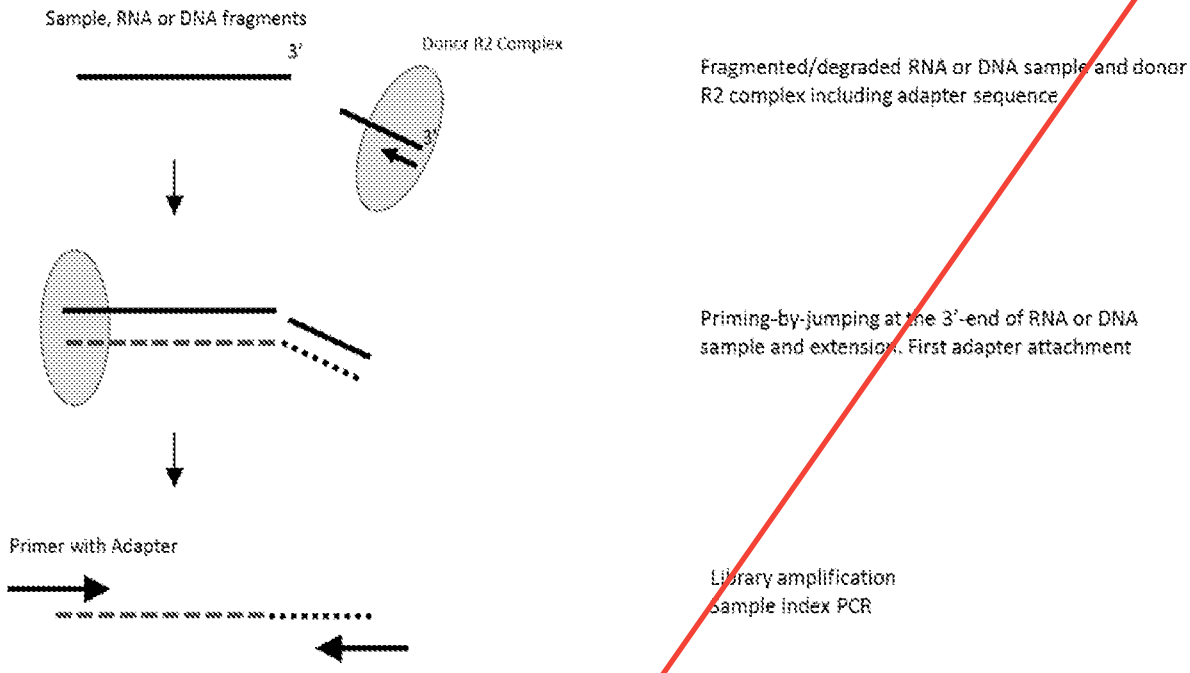


FIGURE 6B

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

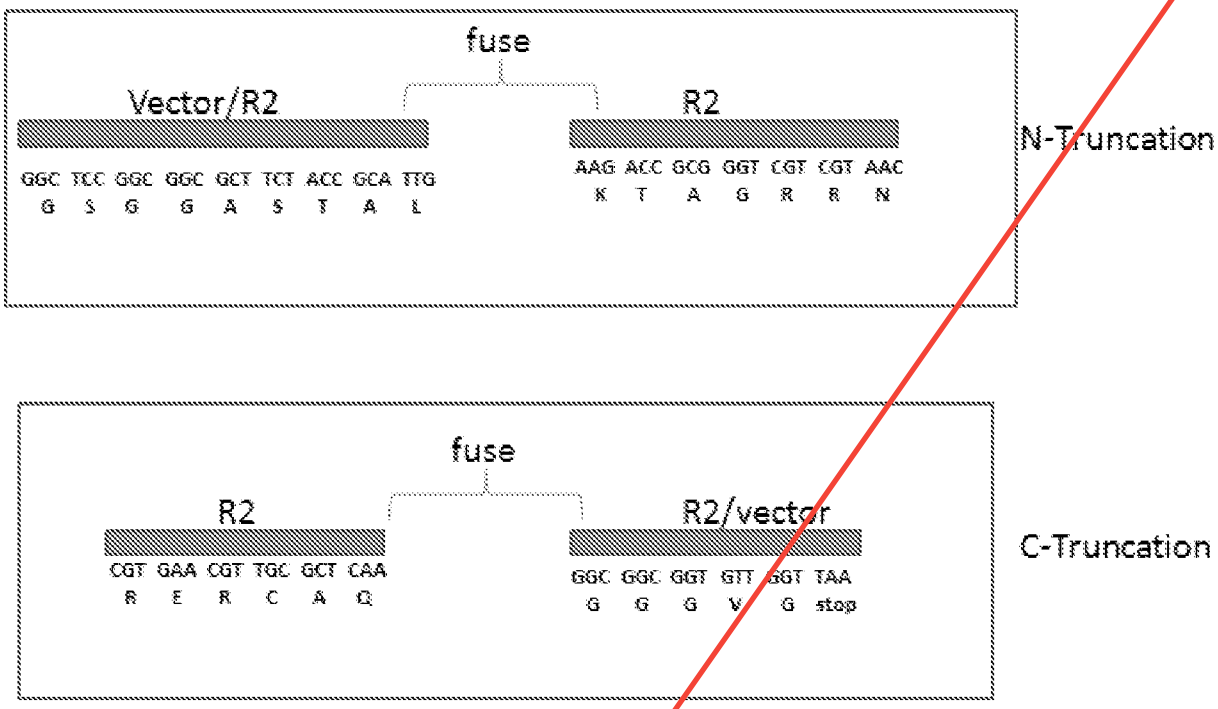


FIGURE 7

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

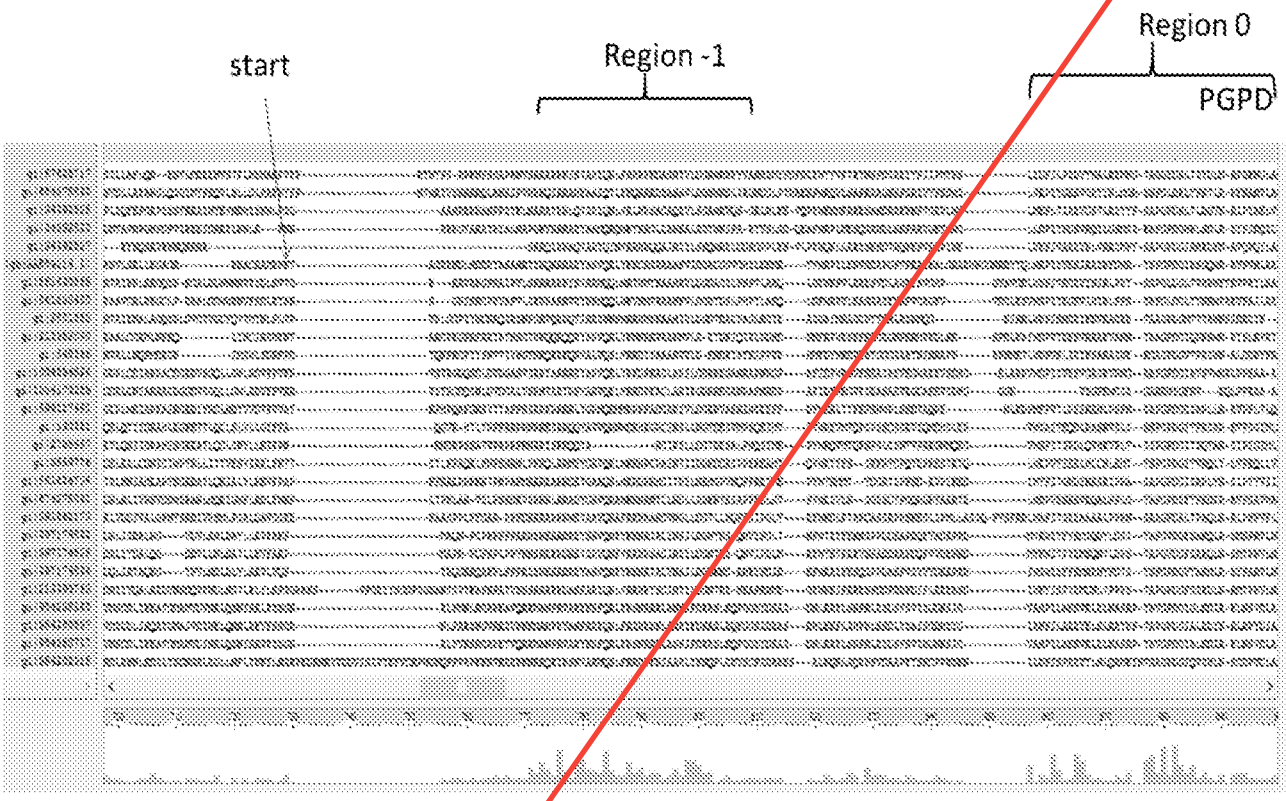


FIGURE 8

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)

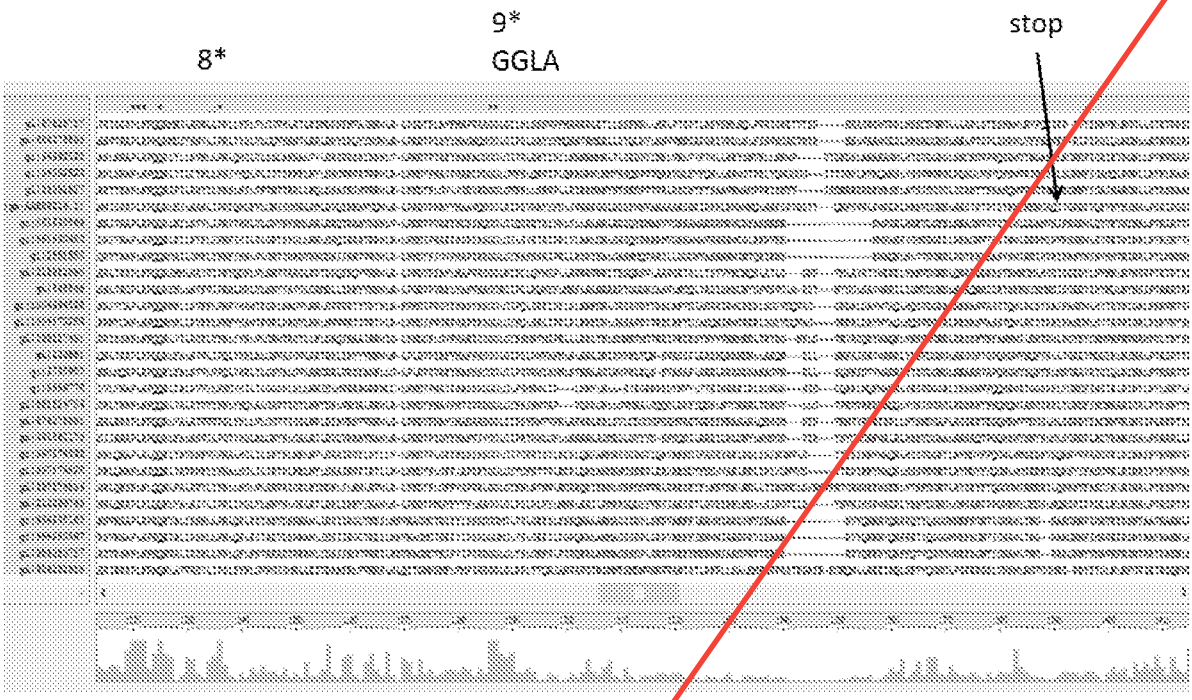


FIGURE 9

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

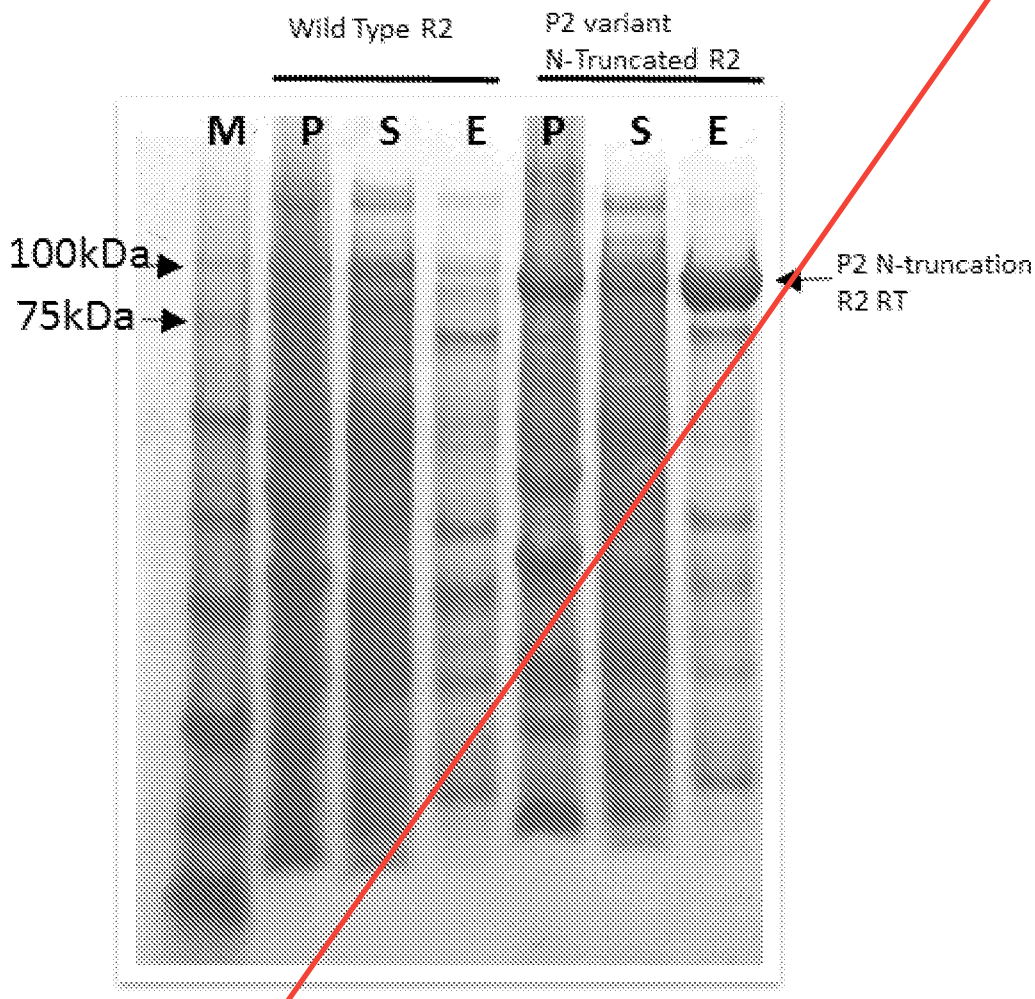


FIGURE 10

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

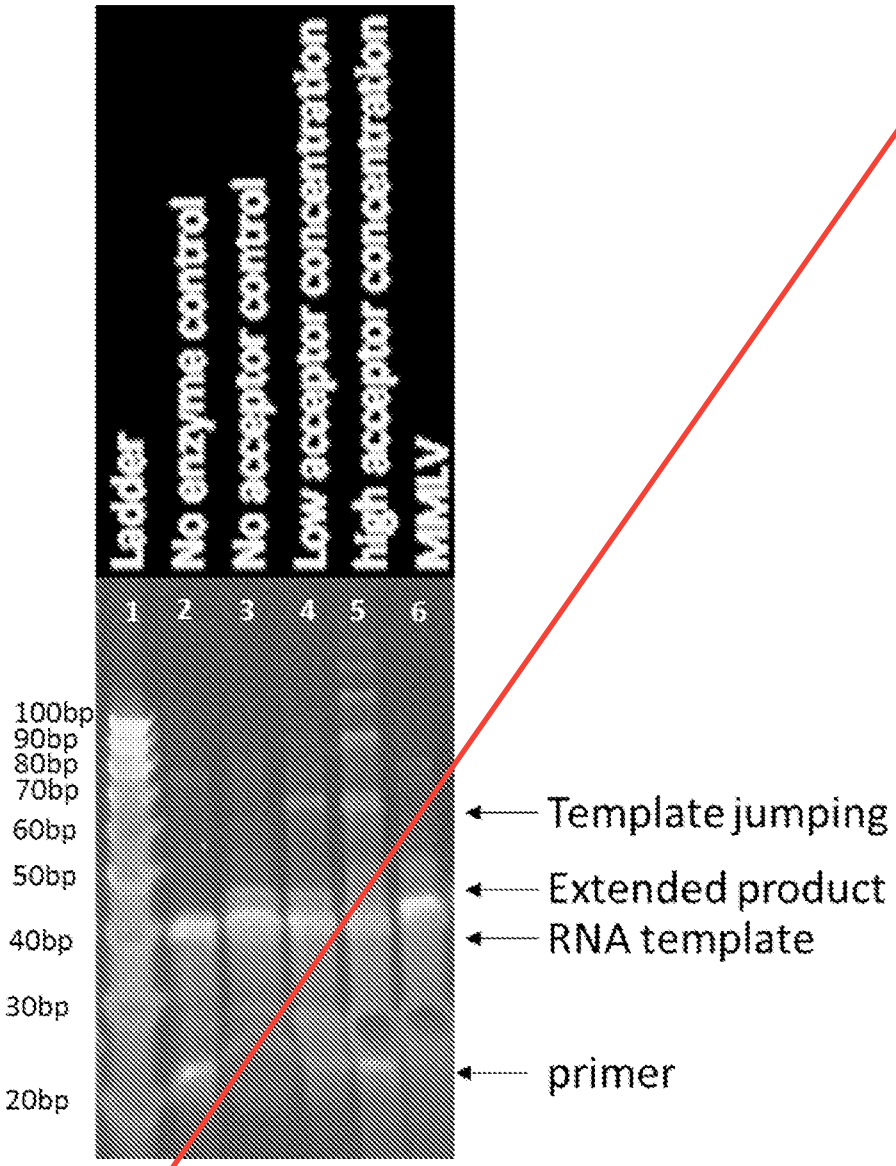


FIGURE 11

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

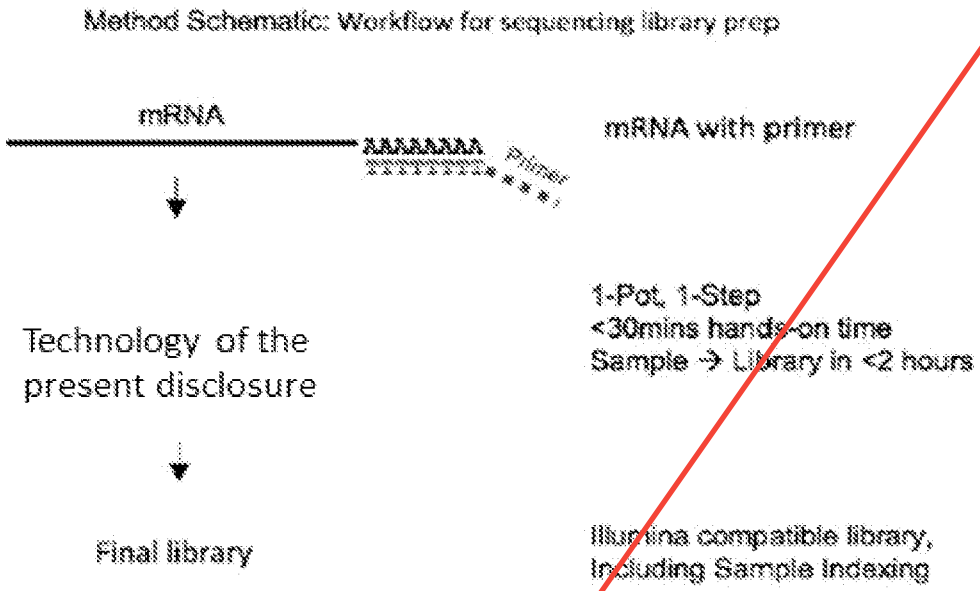


FIGURE 12A

Workflow for sequencing library preparation

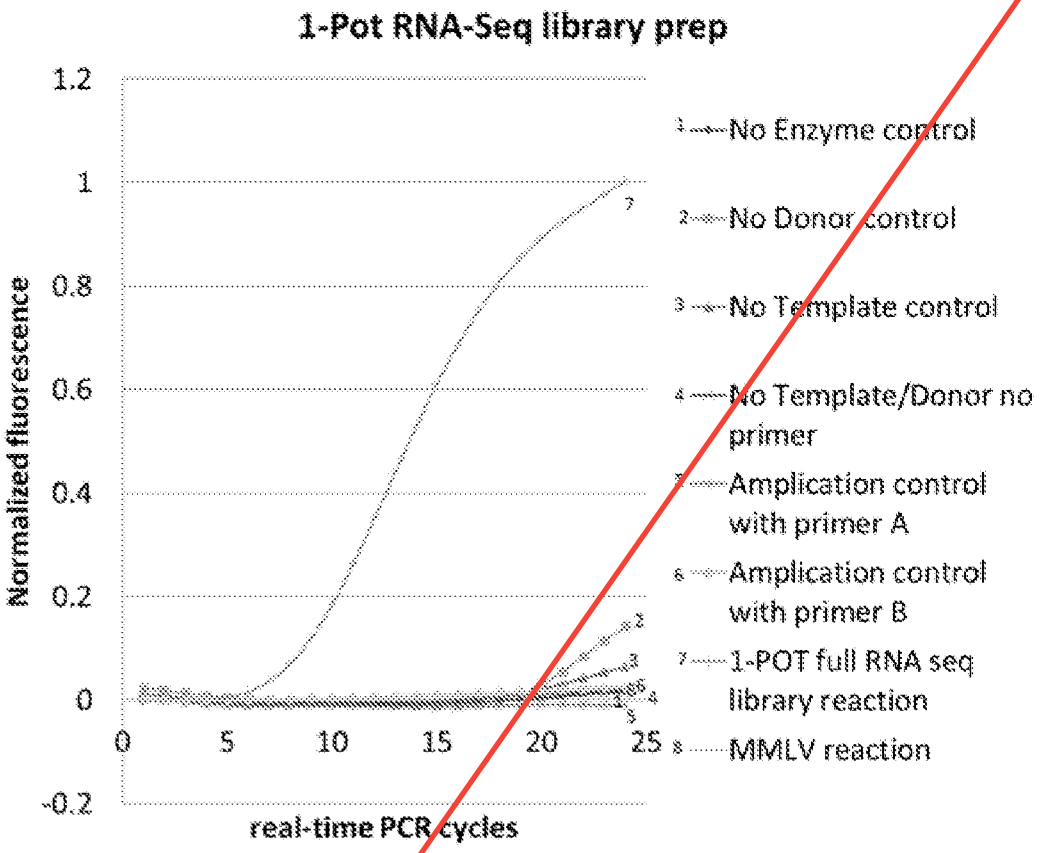


FIGURE 12B

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

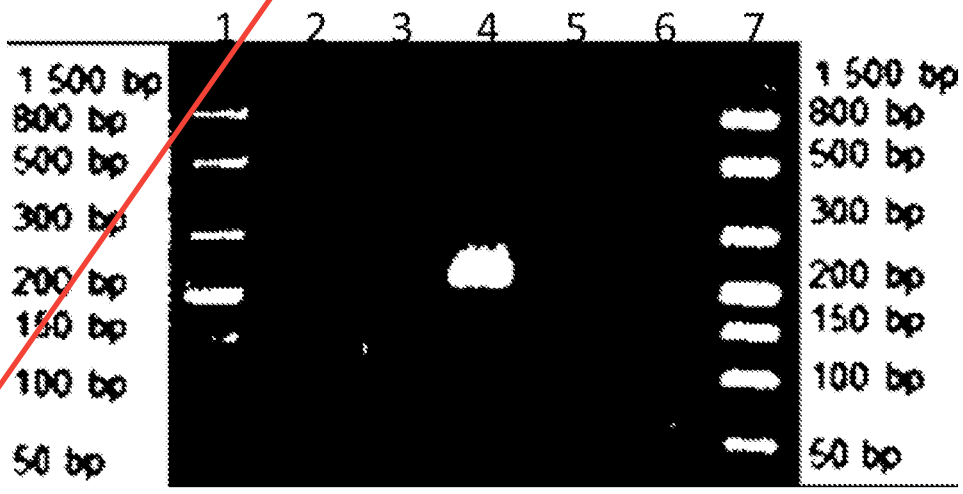


FIGURE 12C

Gel showing amplicon

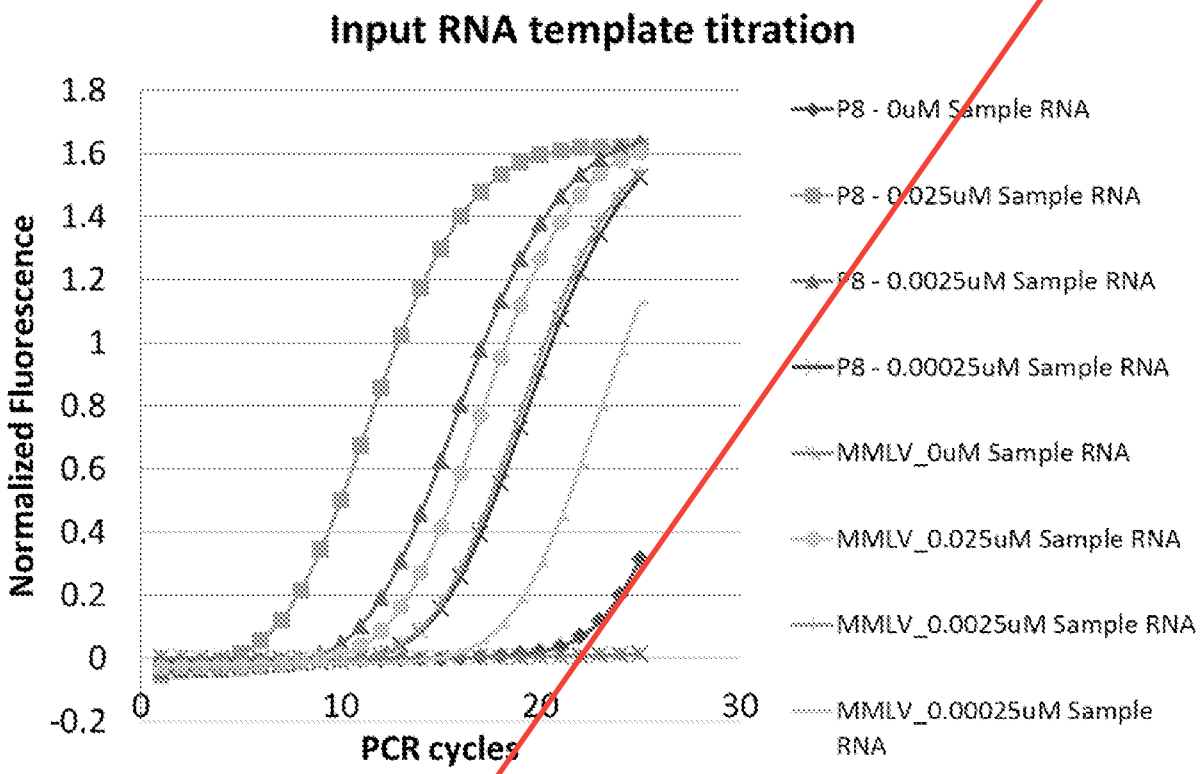


FIGURE 13

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

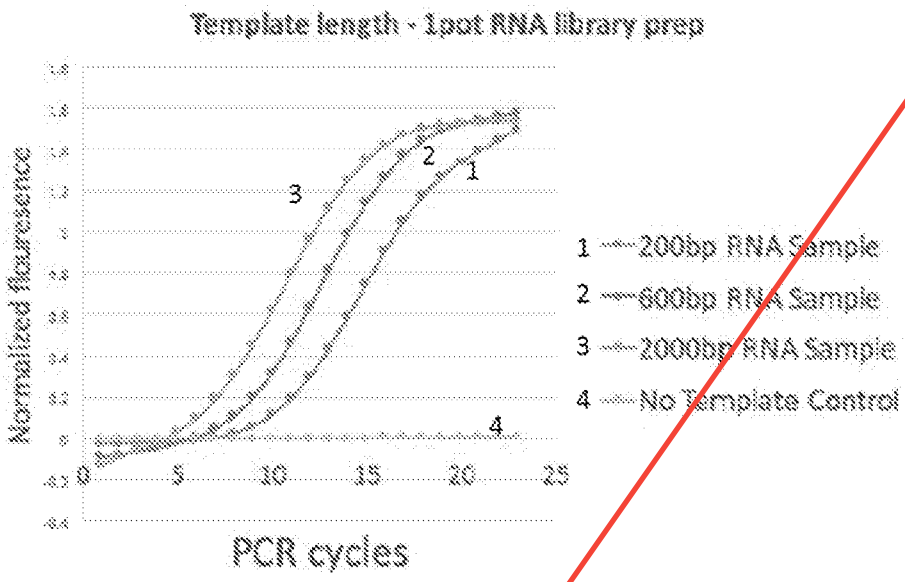


FIGURE 14

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

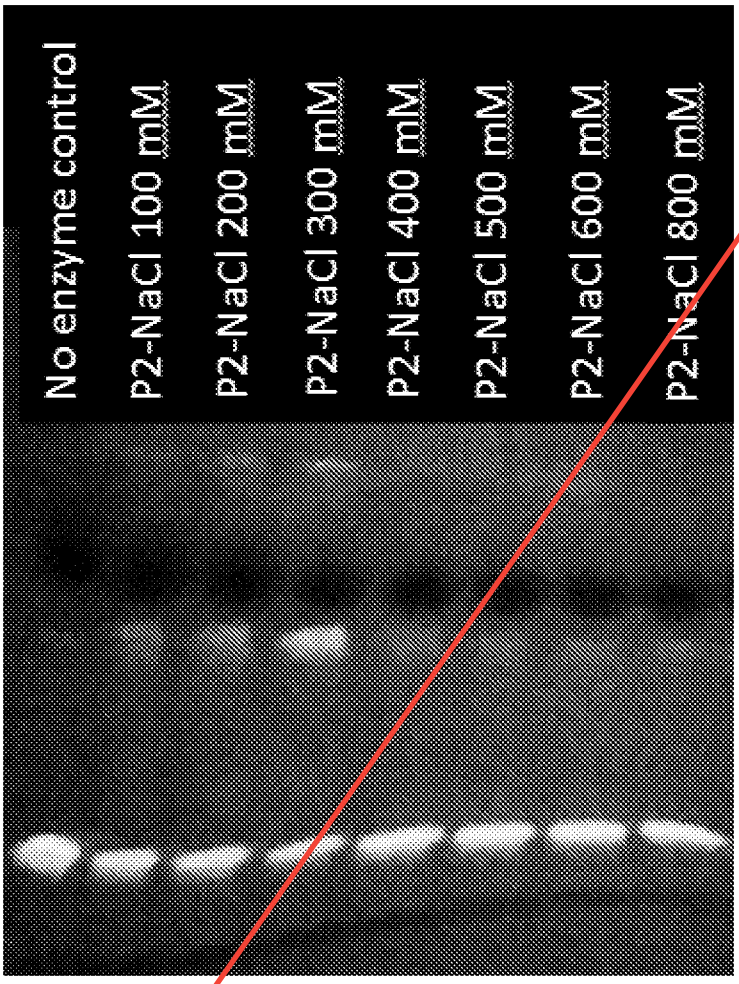


FIGURE 15

Enzyme activity and template jumping is dependent on NaCl concentration

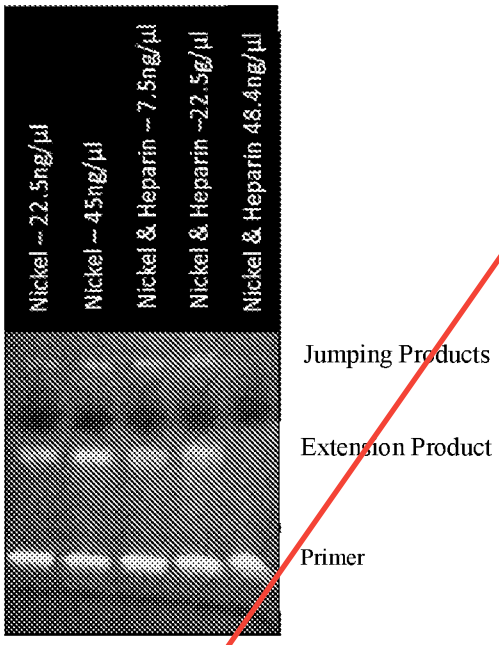


FIGURE 16A

Enzyme activity based on nickel and/or heparin affinity purification

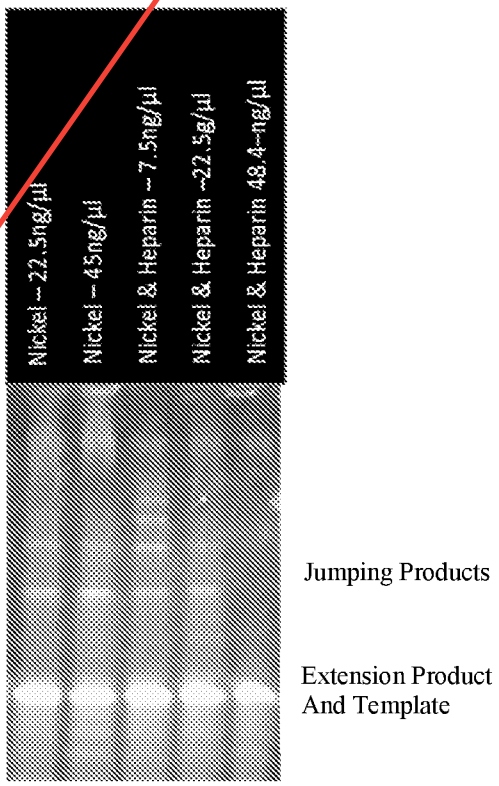


FIGURE 16B

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

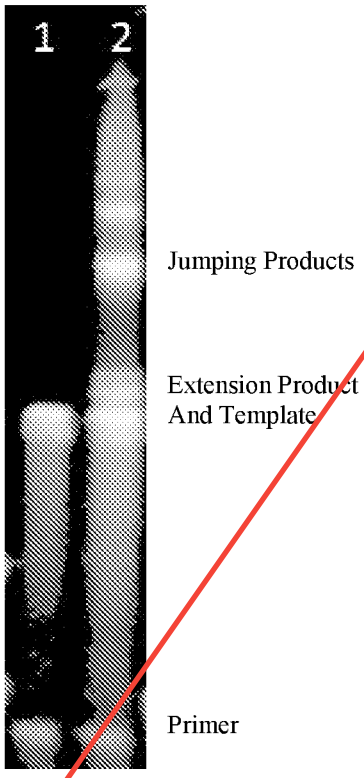


FIGURE 17

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)

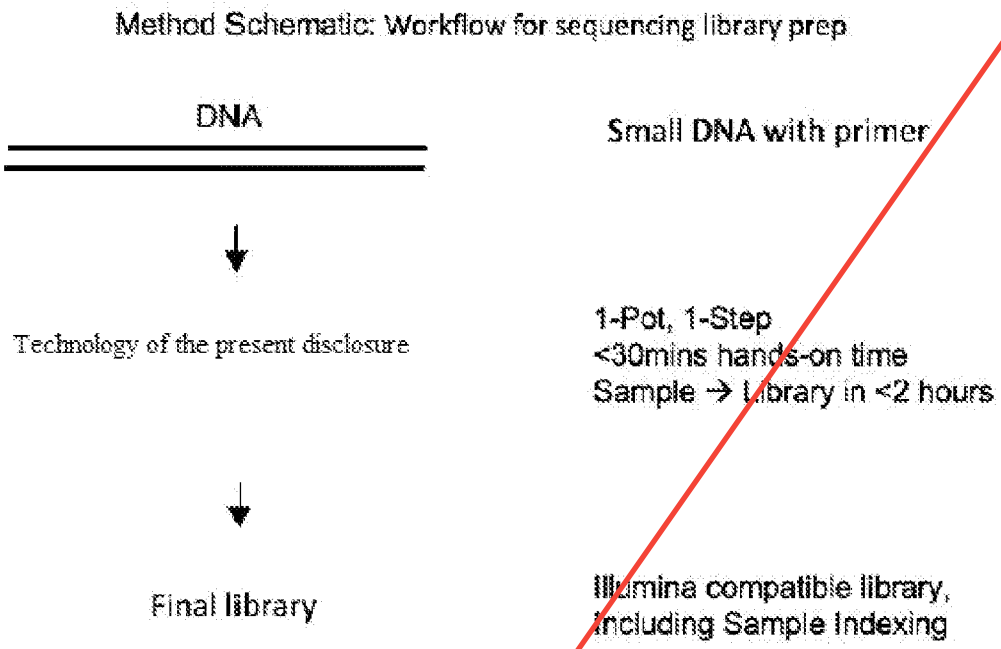


FIGURE 18A

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

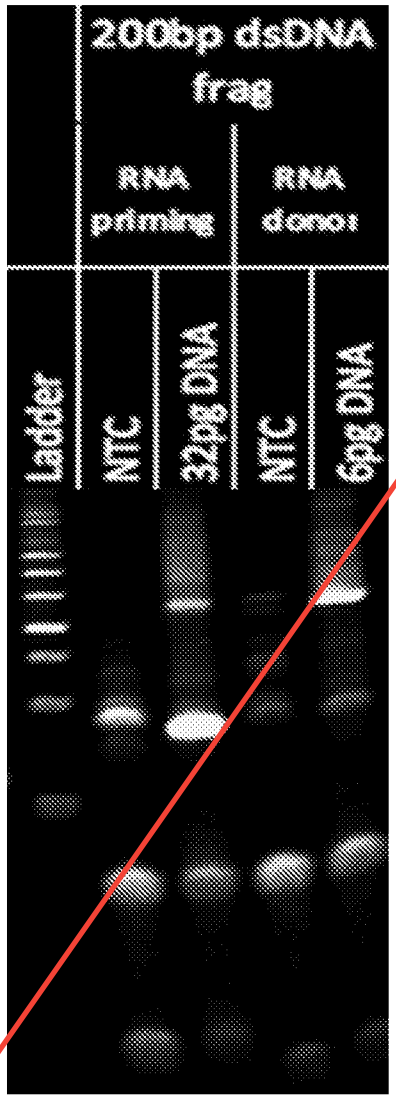


FIGURE 18B

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

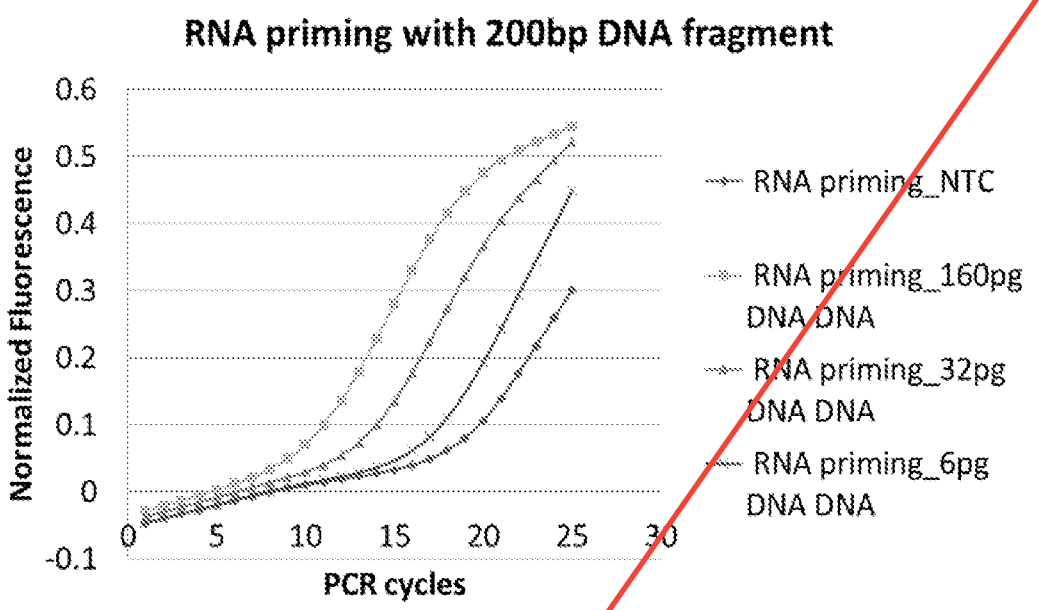


FIGURE 18C

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

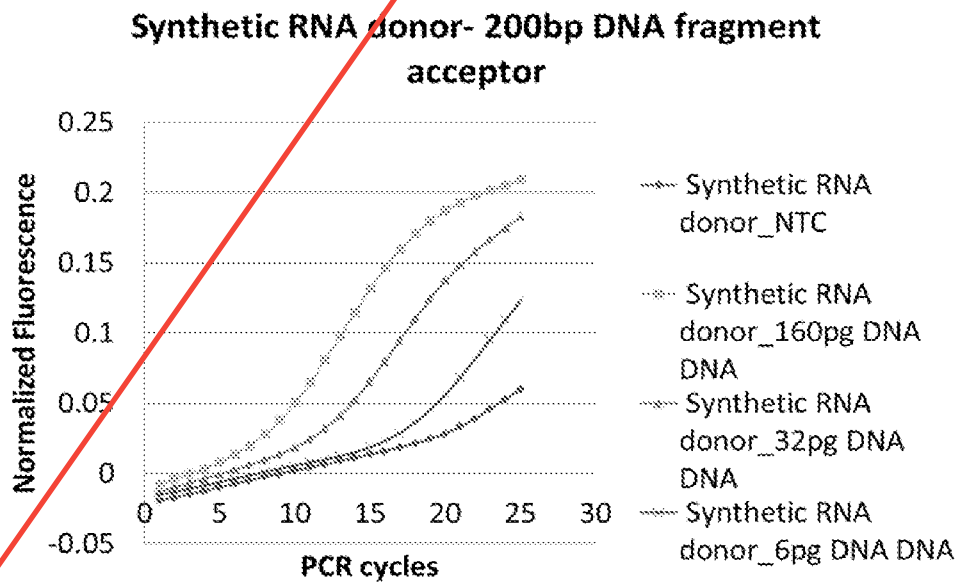


FIGURE 18D

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

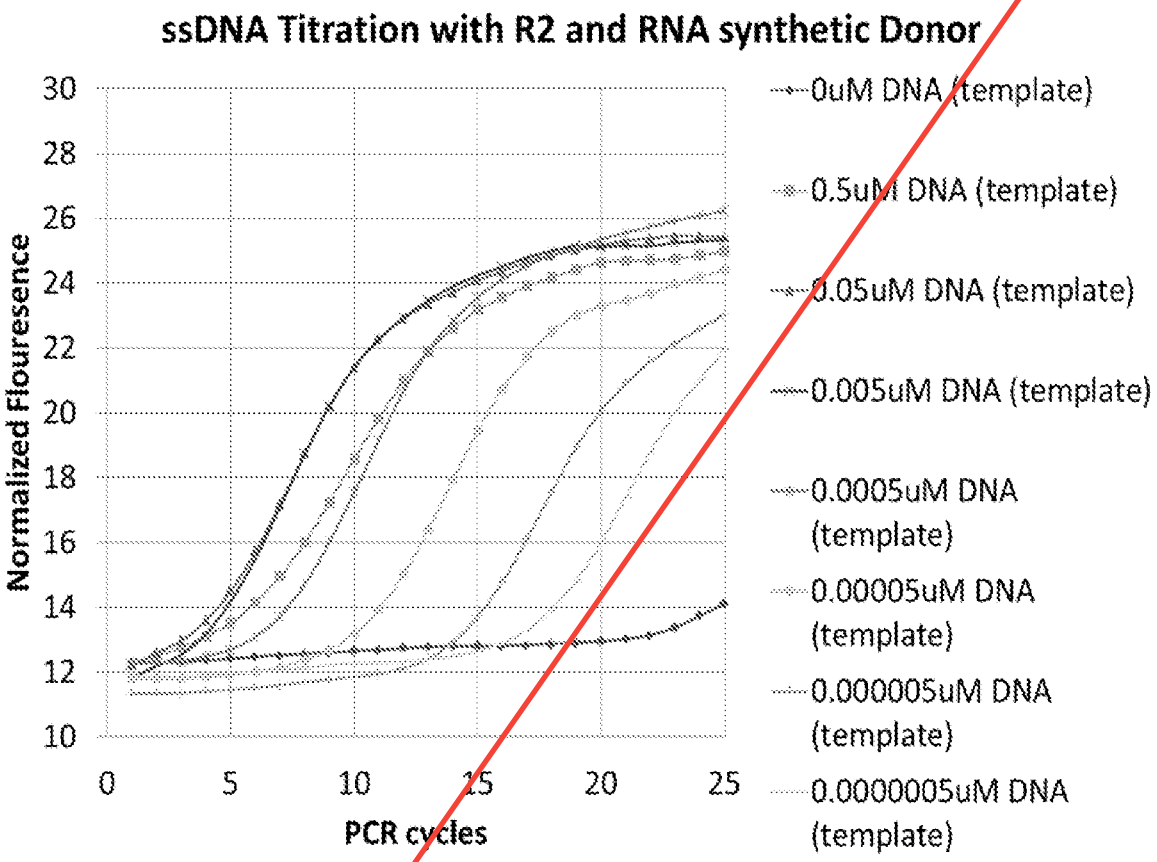


FIGURE 19

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

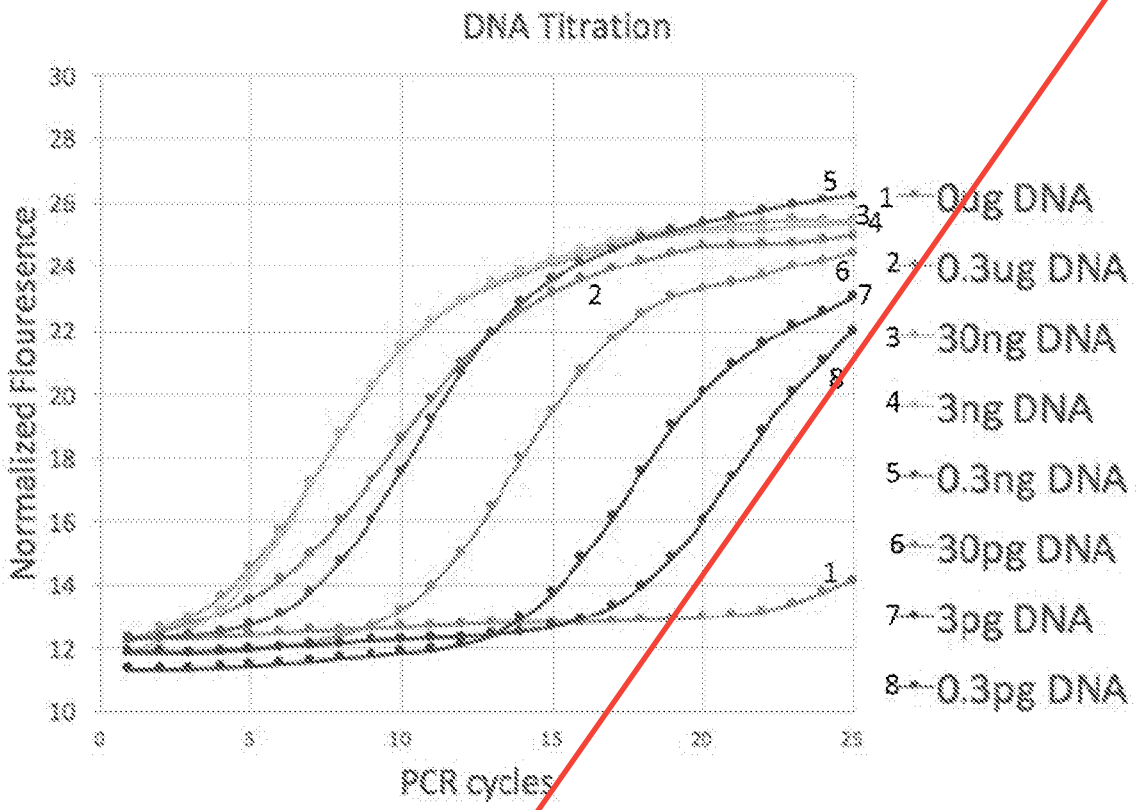


FIGURE 20

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

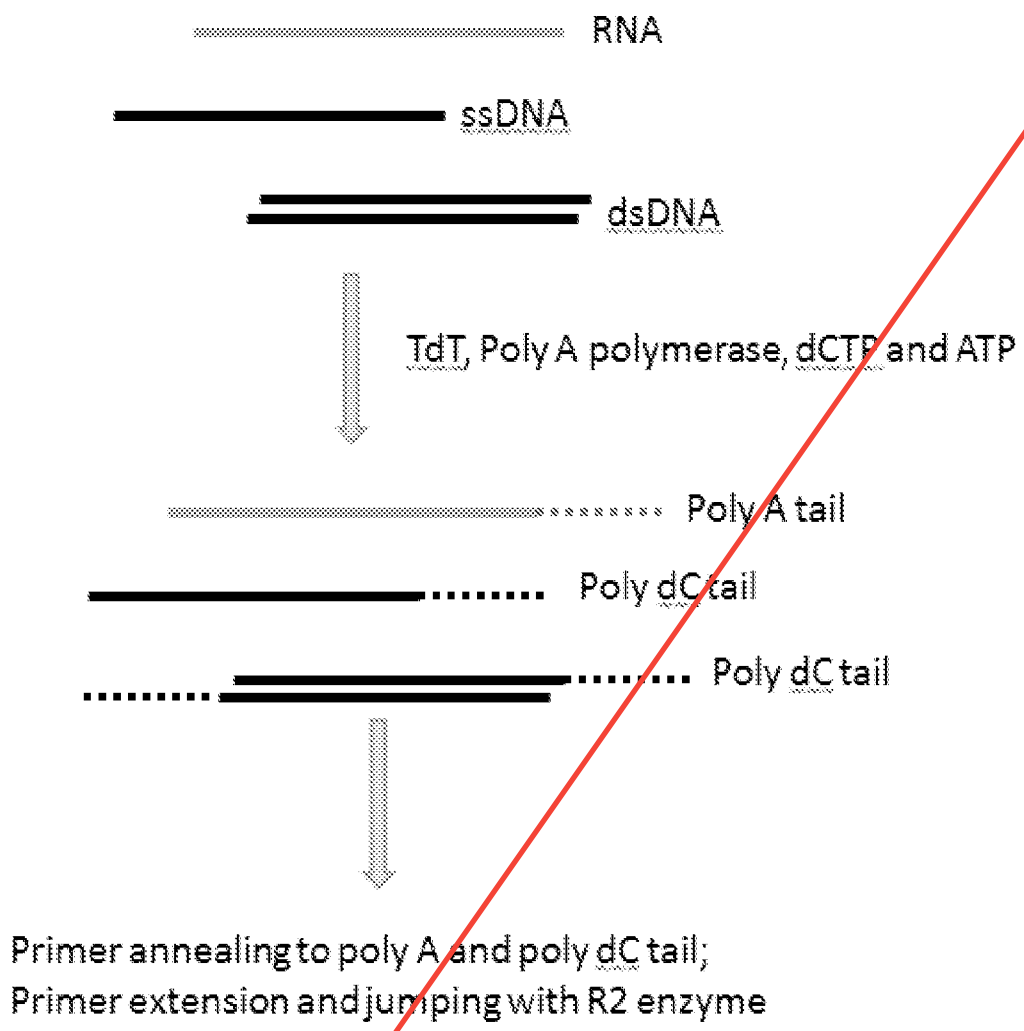


FIGURE 21

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

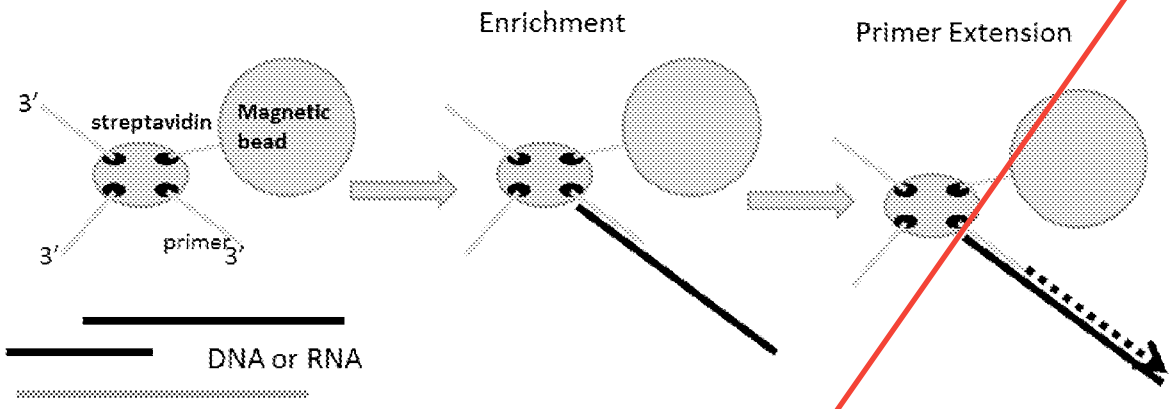


FIGURE 22A

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).

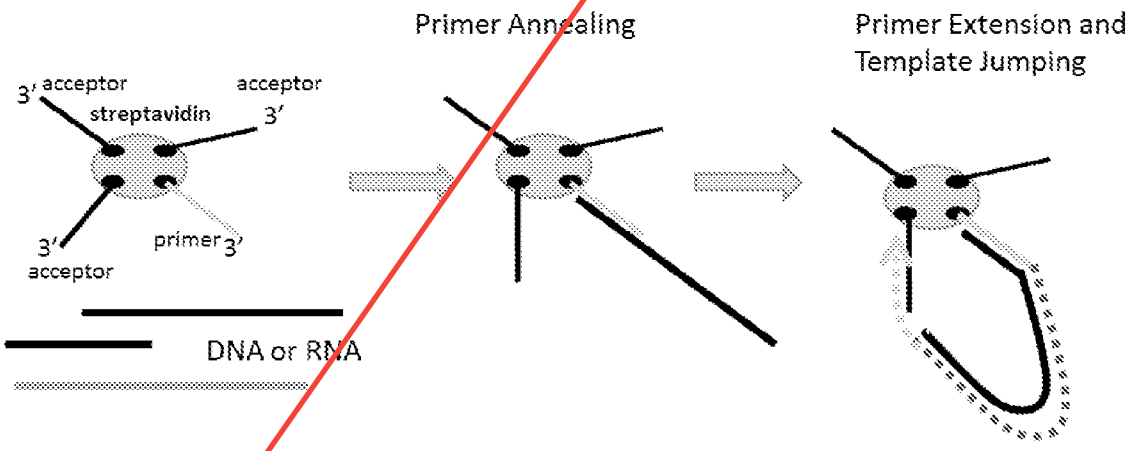


FIGURE 22B

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.

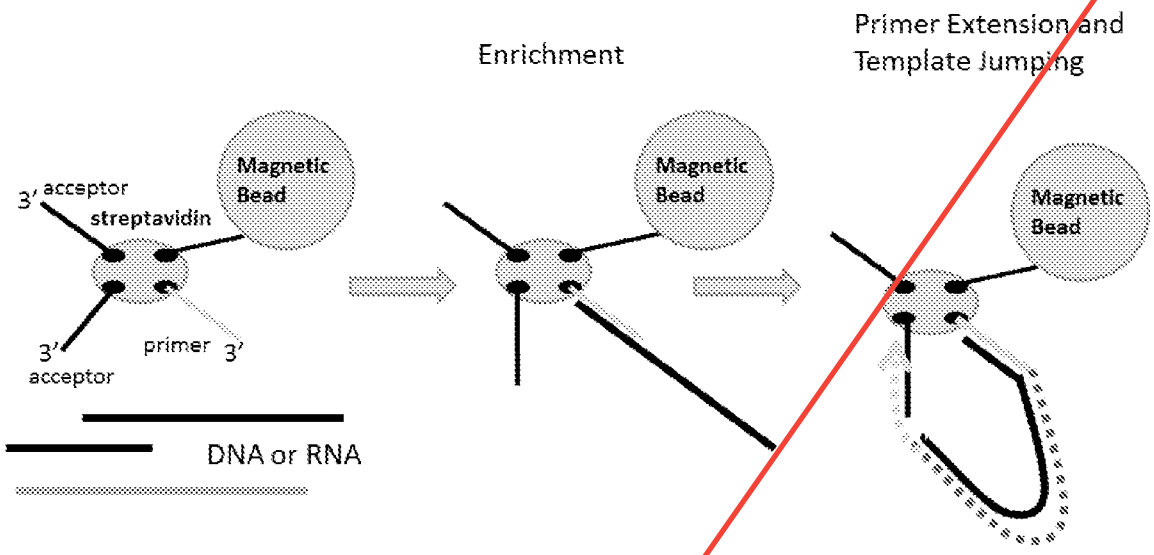


FIGURE 22C

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

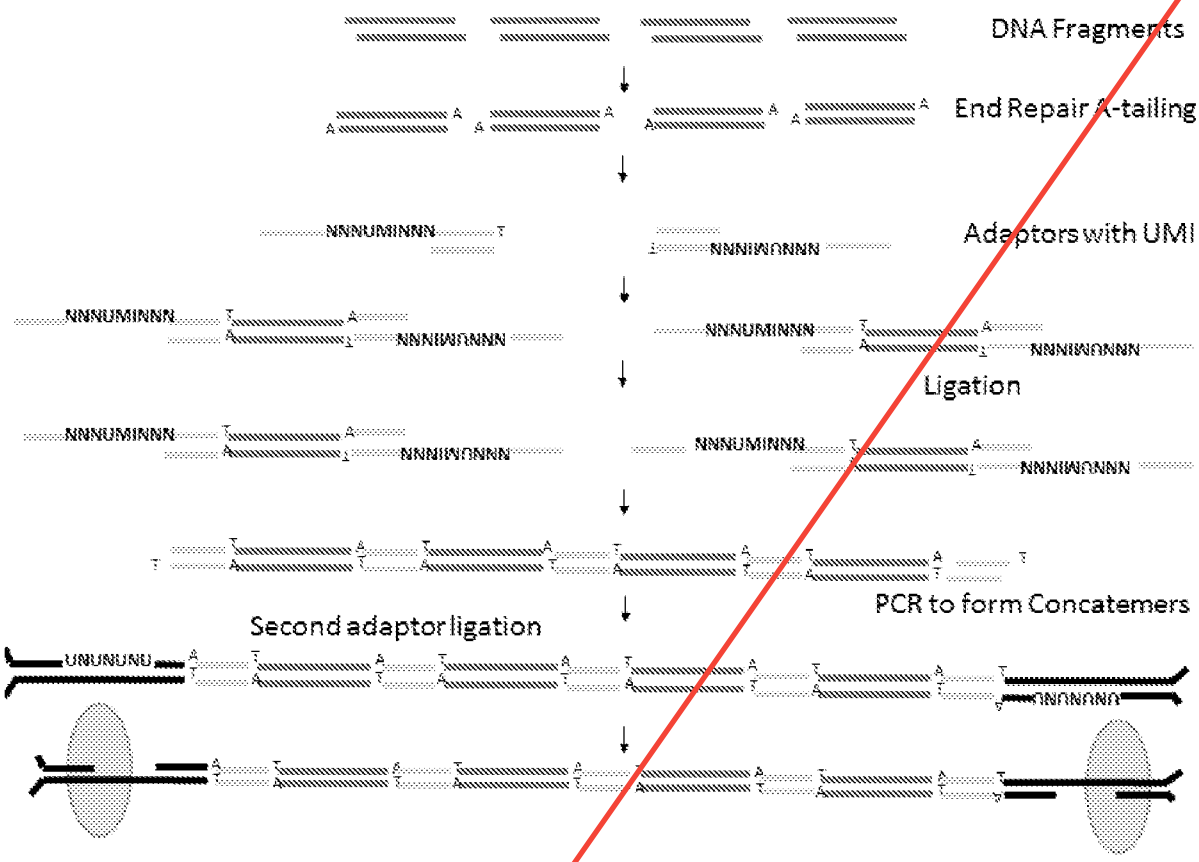


FIGURE 23A

Template Concatemerization

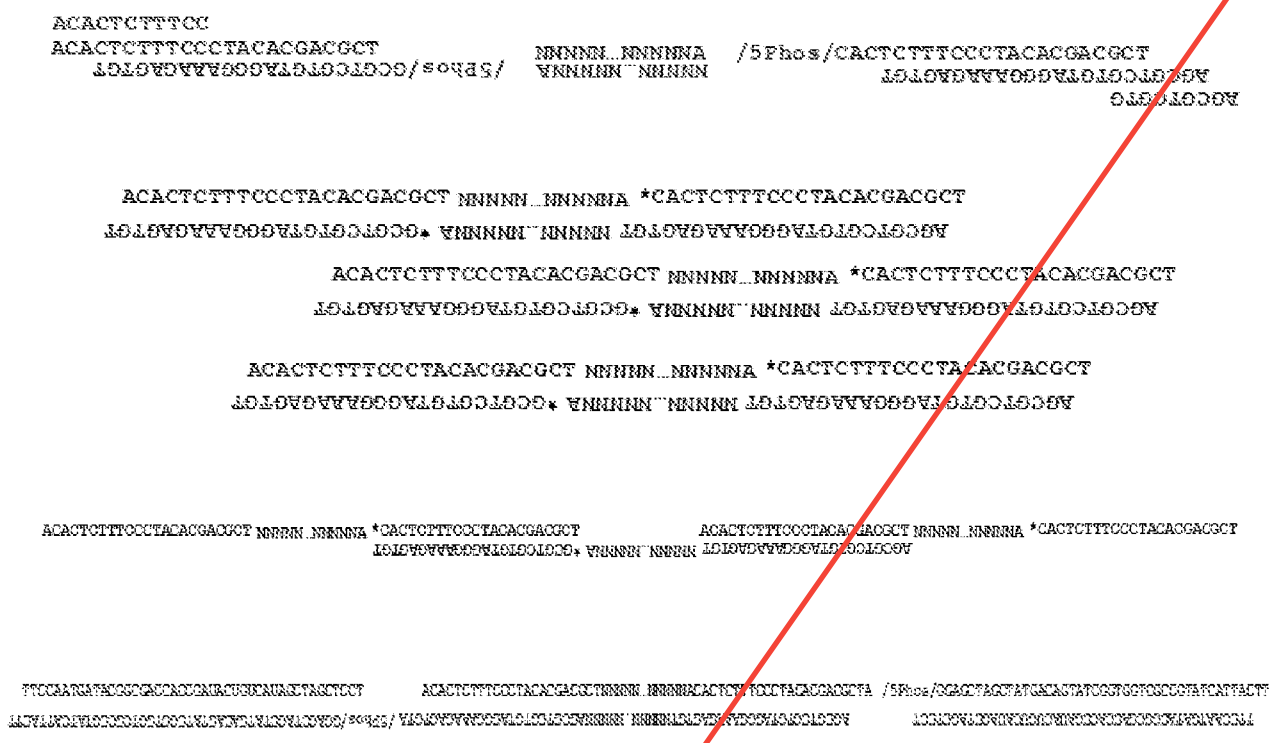


FIGURE 23B

Product of a concatemerization that includes specific adaptors on both ends

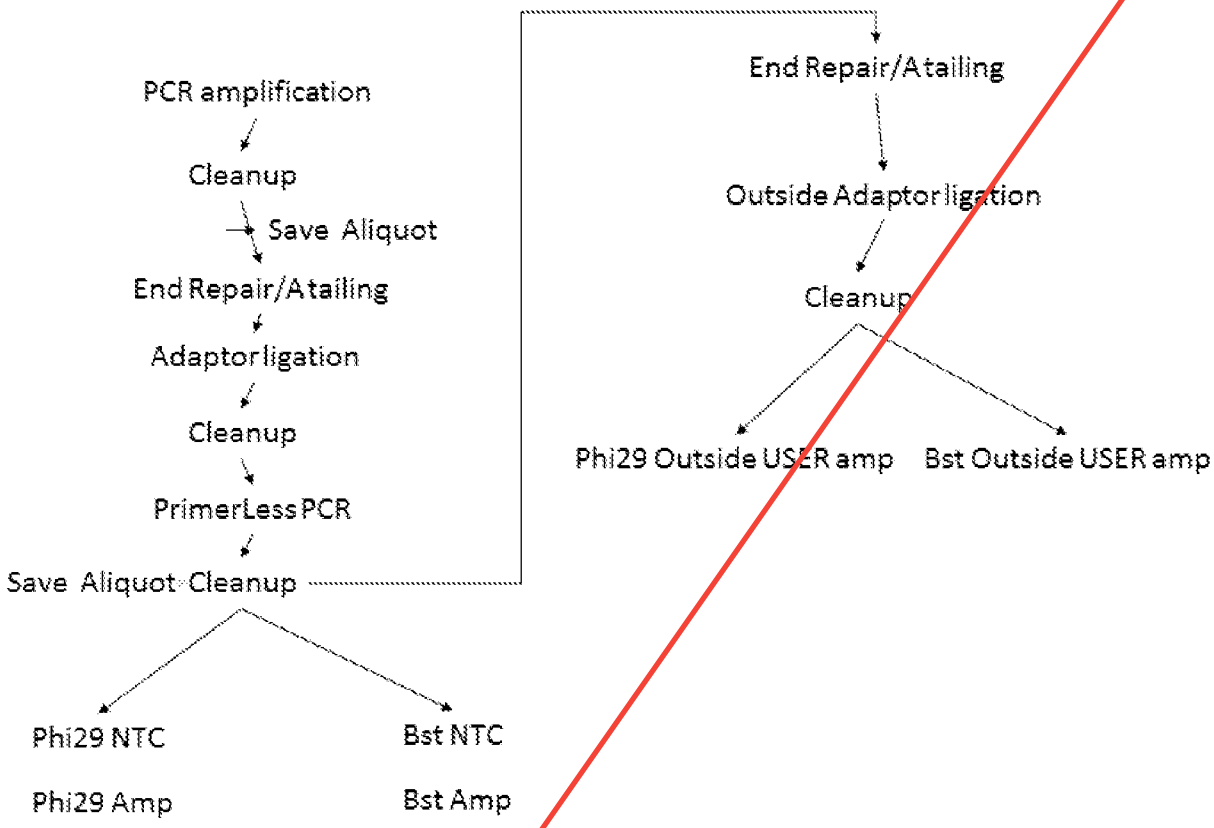


FIGURE 24A

Workflow of template concatemerization

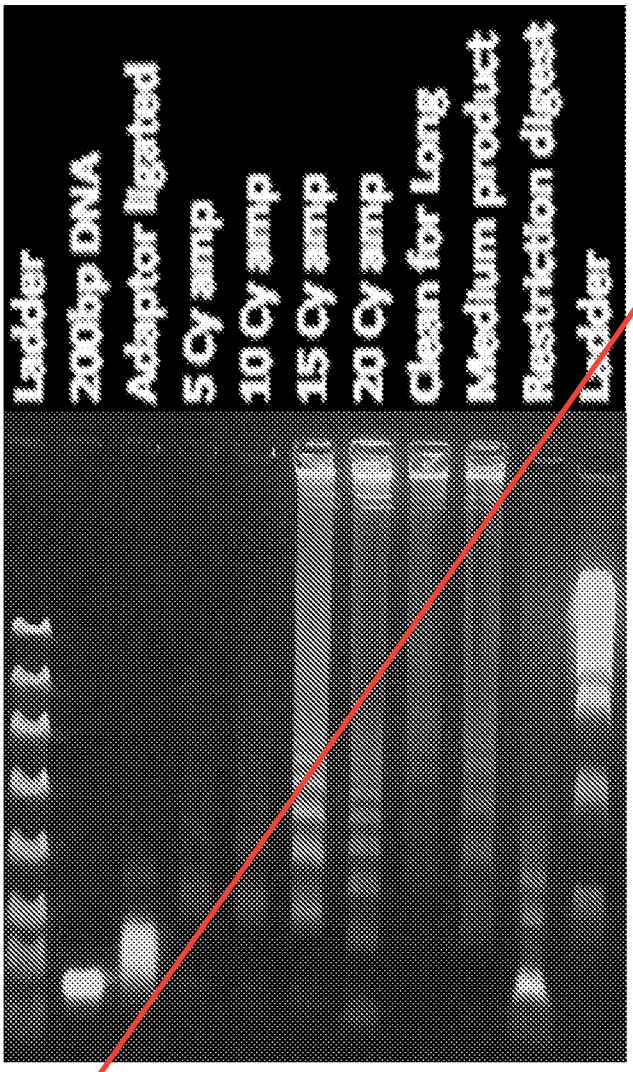


FIGURE 24B

Gel showing the concatemerization of a 200bp DNA fragment

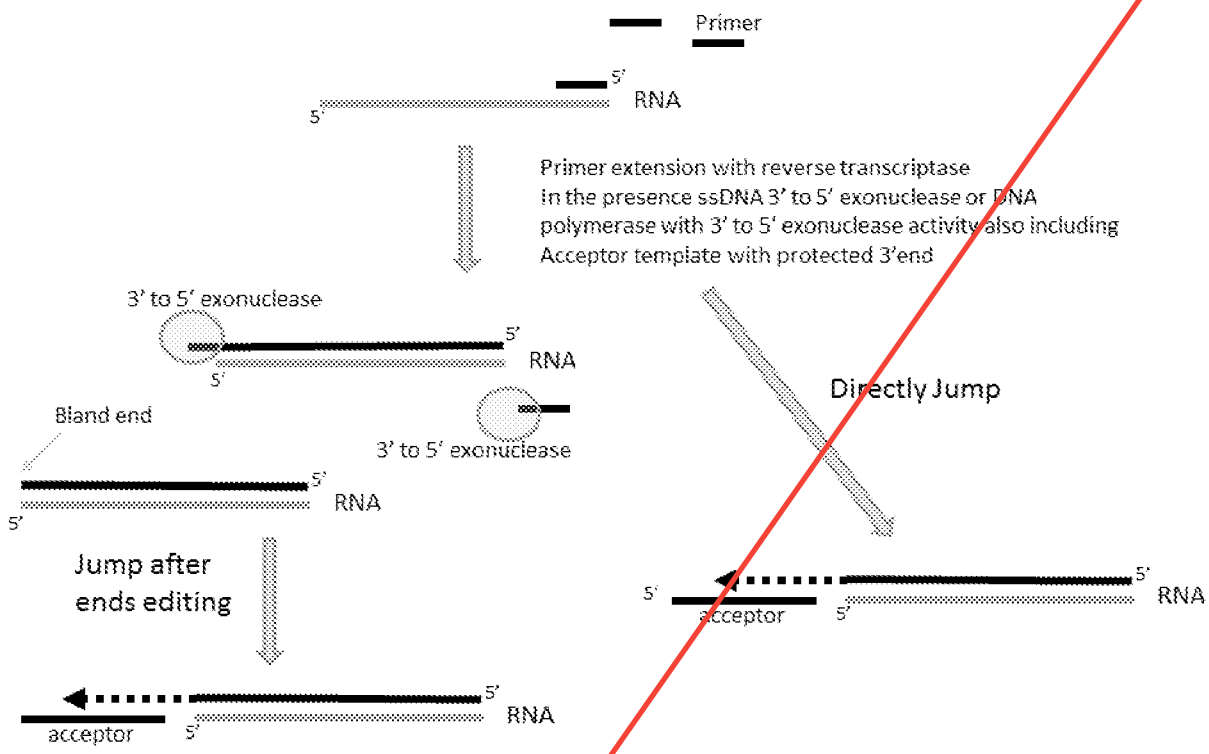


FIGURE 25

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

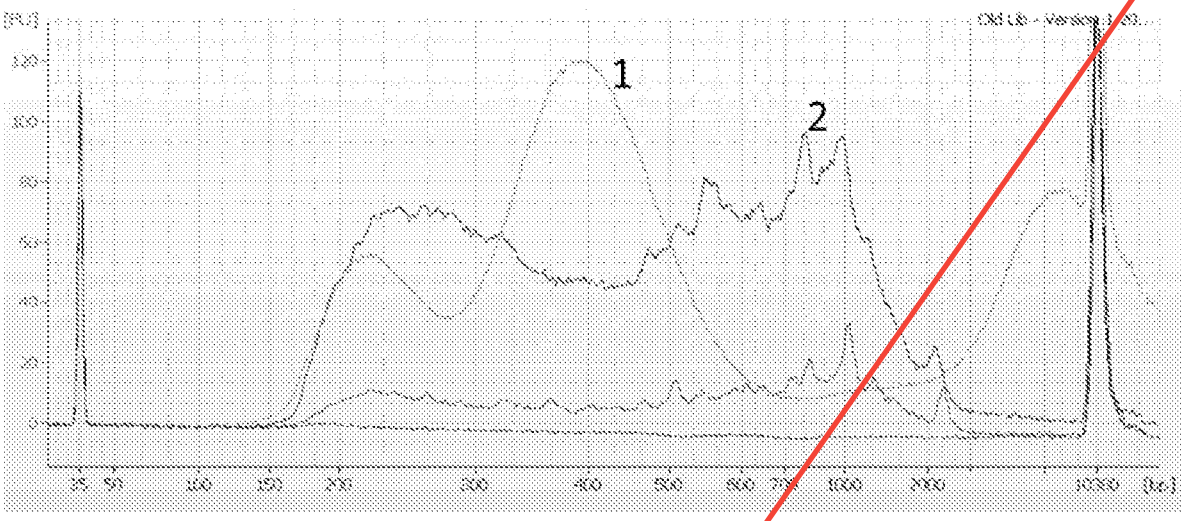


FIGURE 26

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

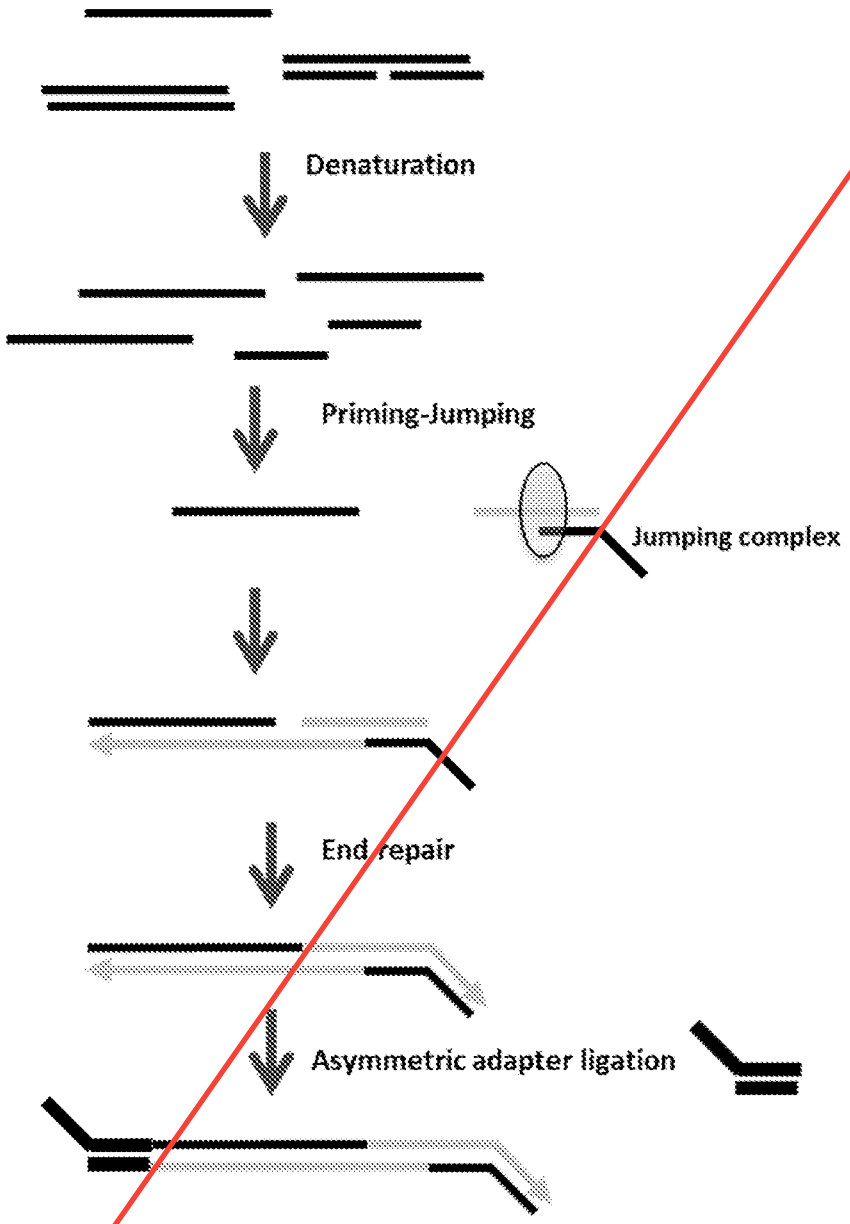


FIGURE 27

Method of generating cfDNA library

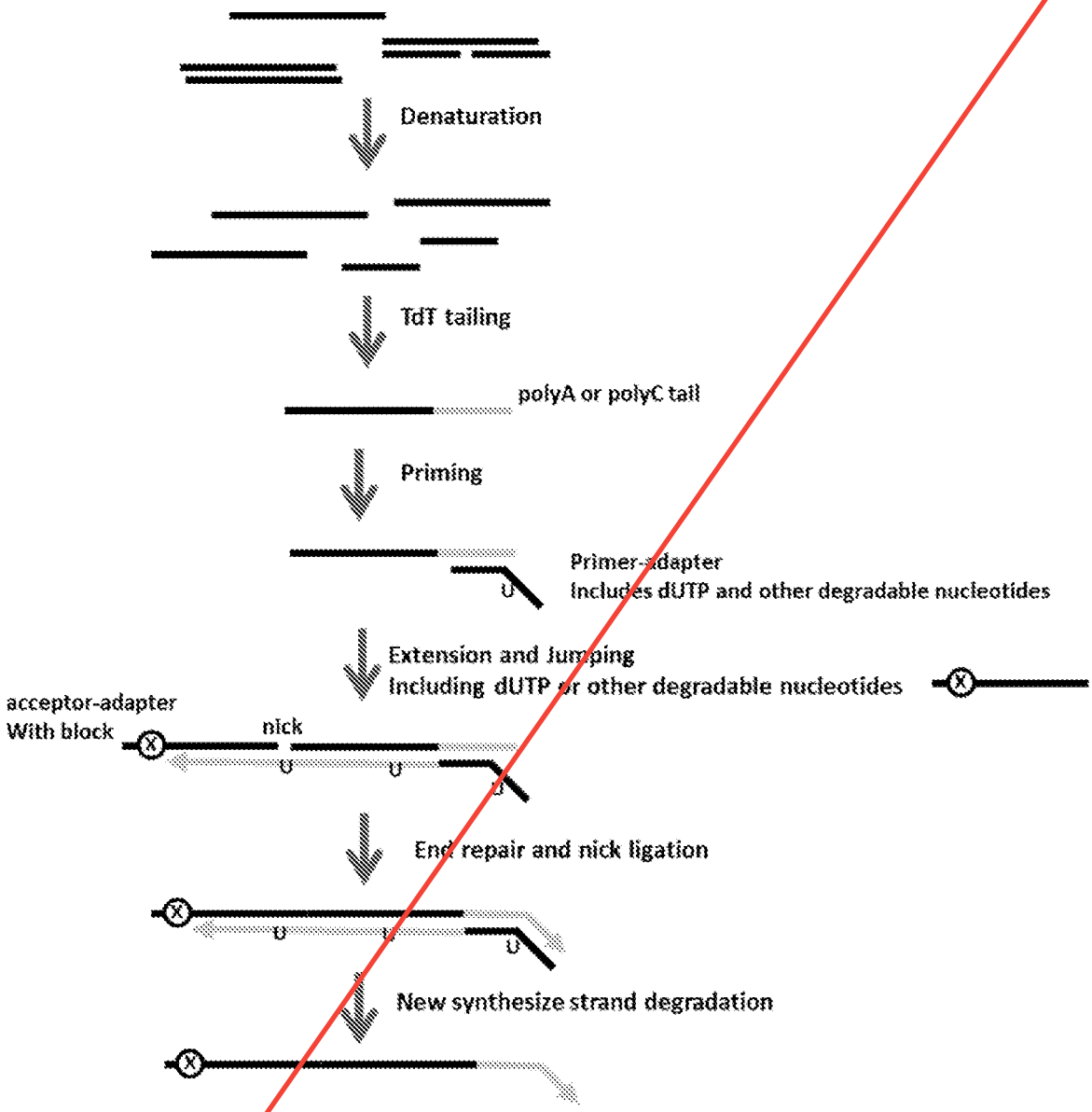


FIGURE 28

Method of generating cfDNA library

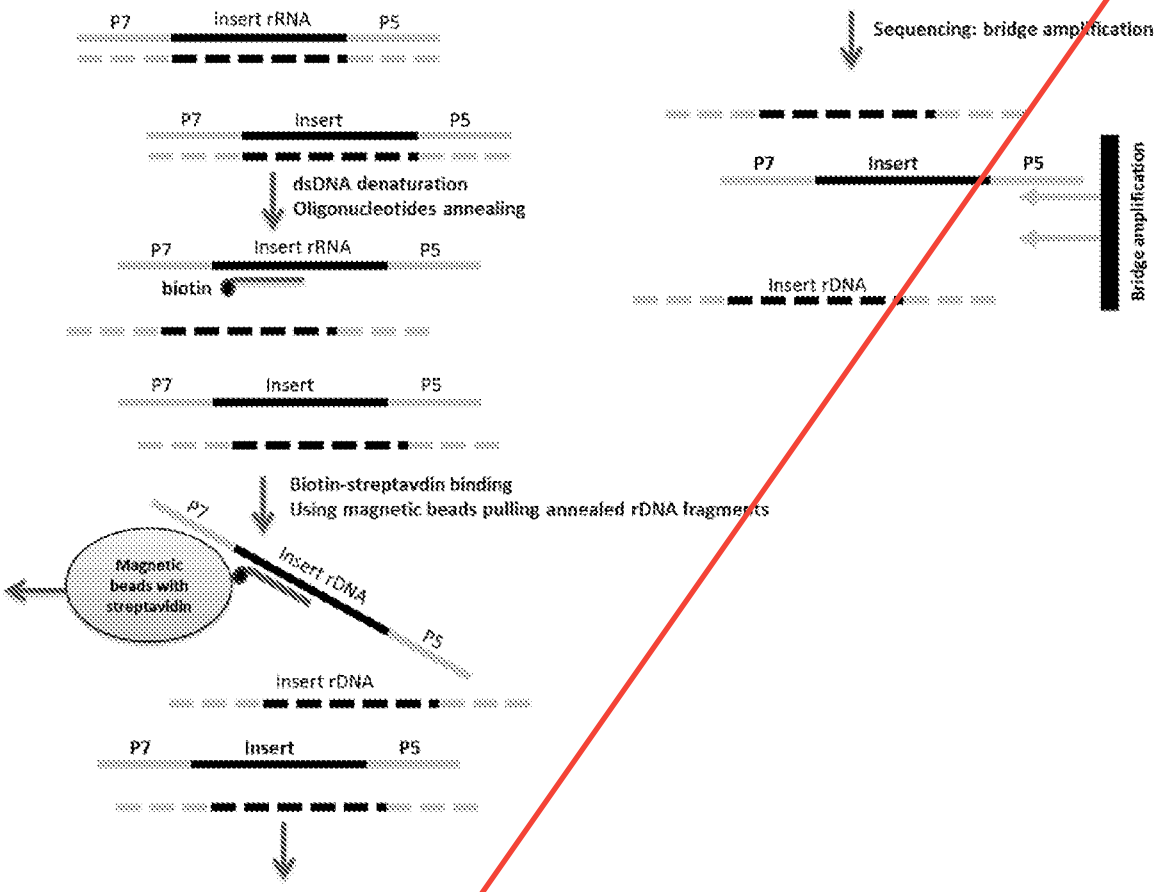


FIGURE 29

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

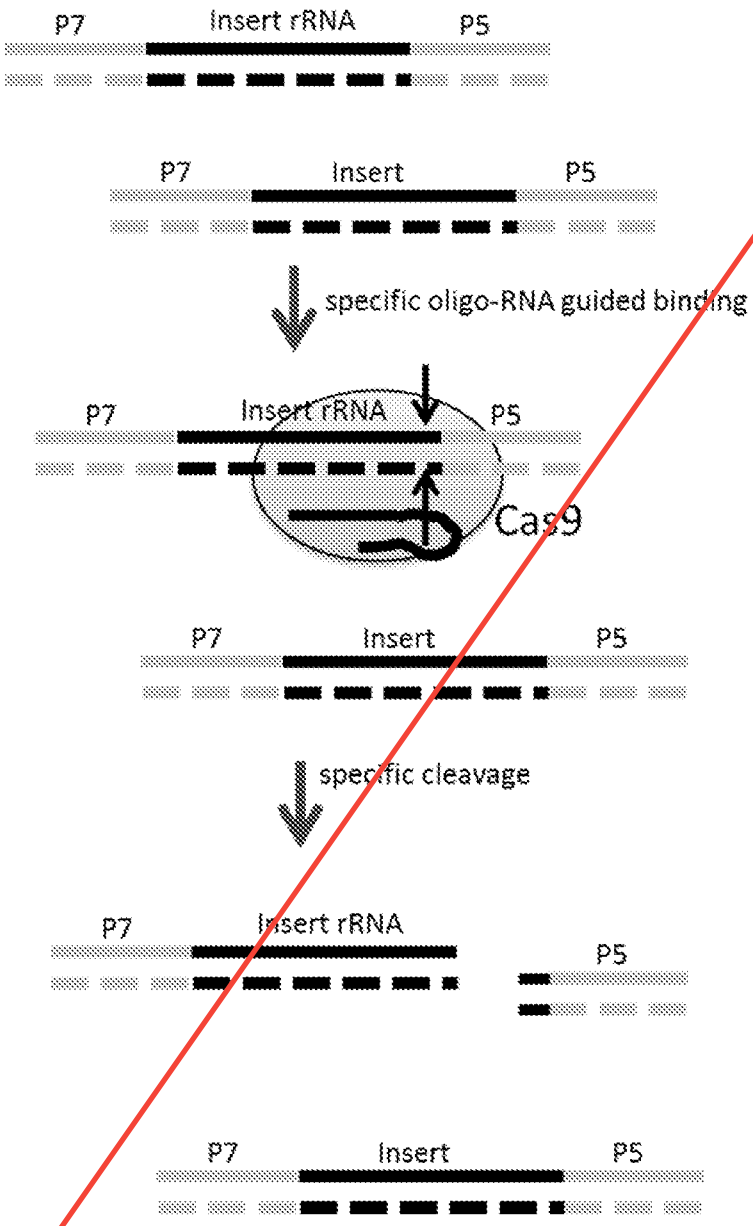


FIGURE 30

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

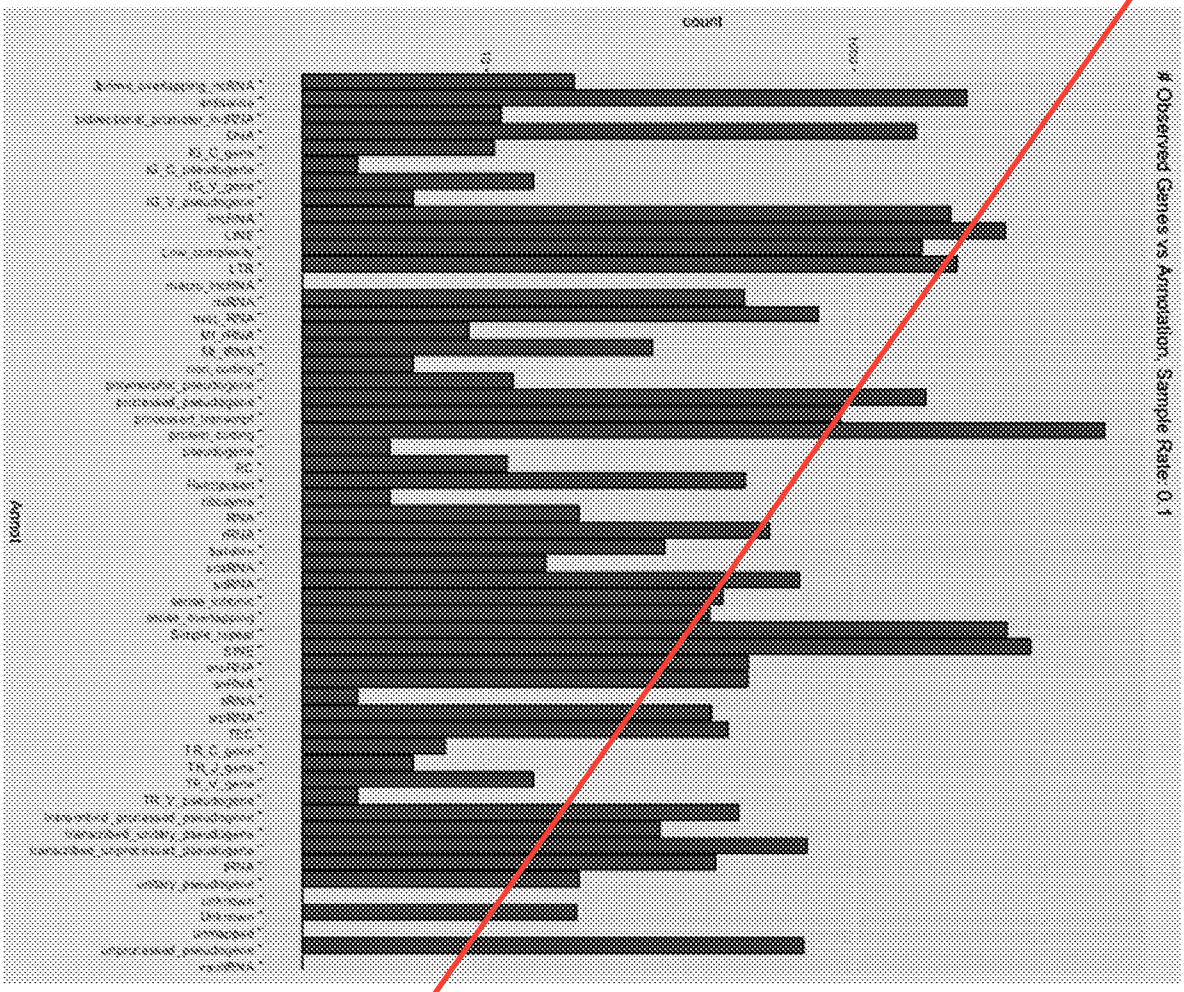


FIGURE 31

Technology capable of targeting all species of RNA simultaneously

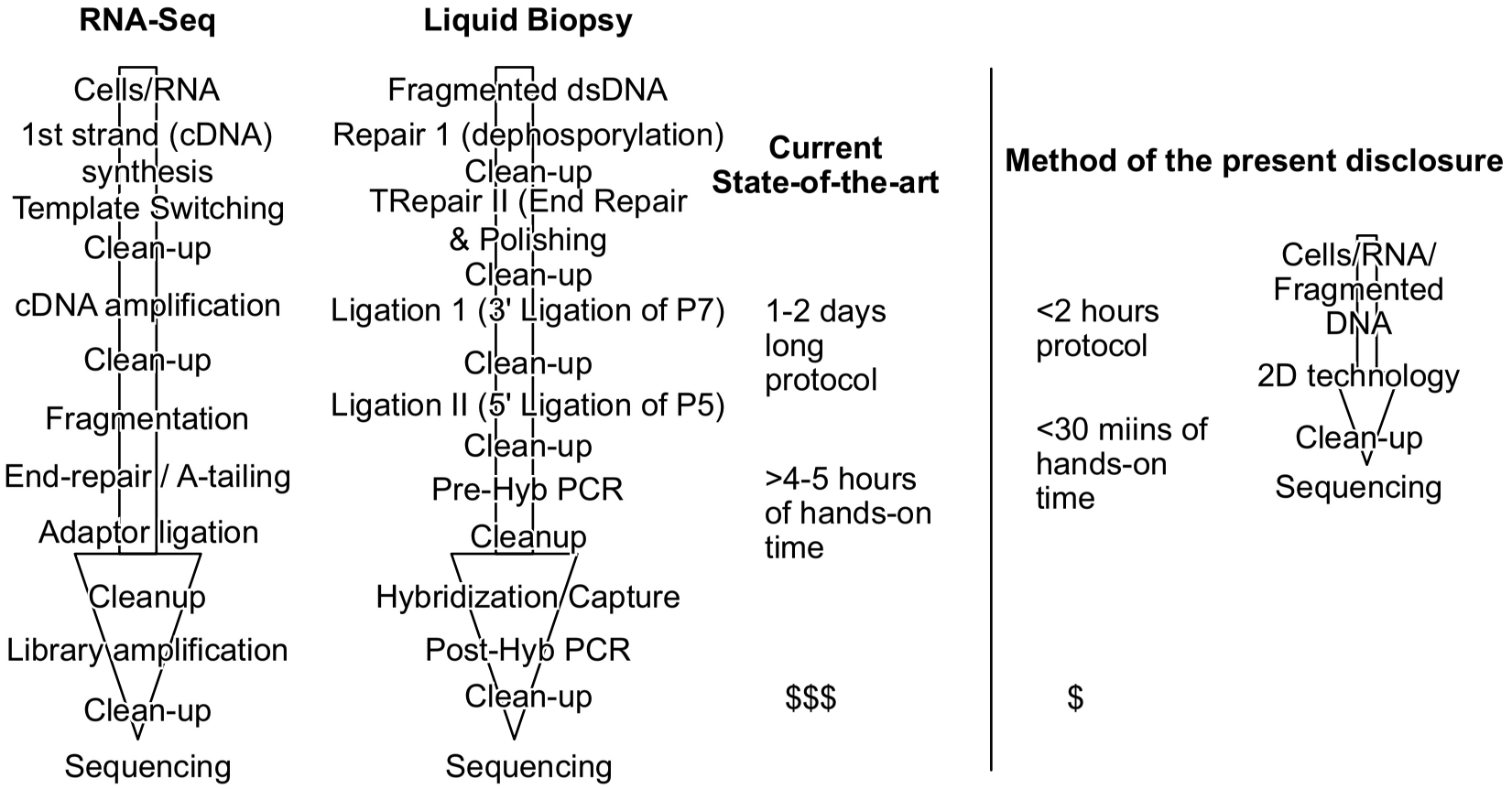


FIG. 1
Workflow comparison

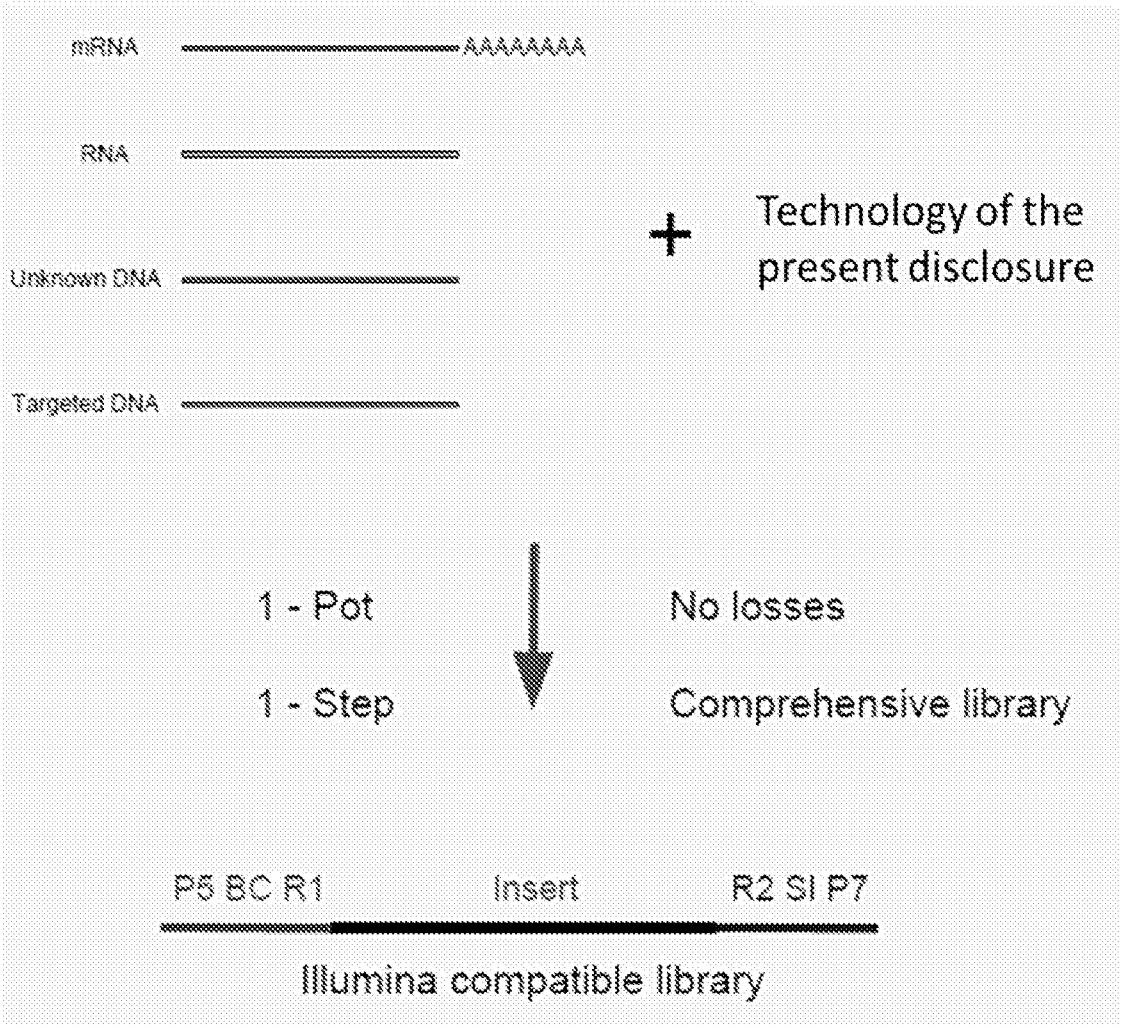


FIGURE 2

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

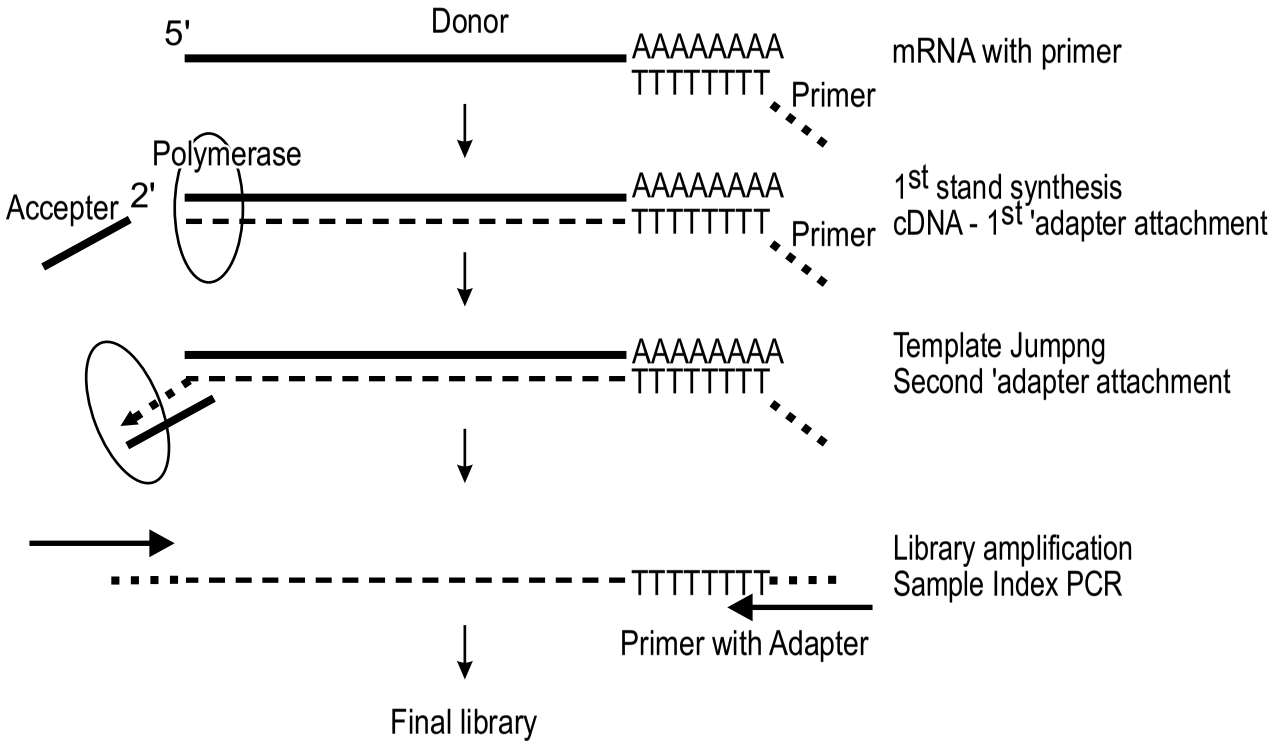


FIG. 3

Example of a workflow for constructing a library

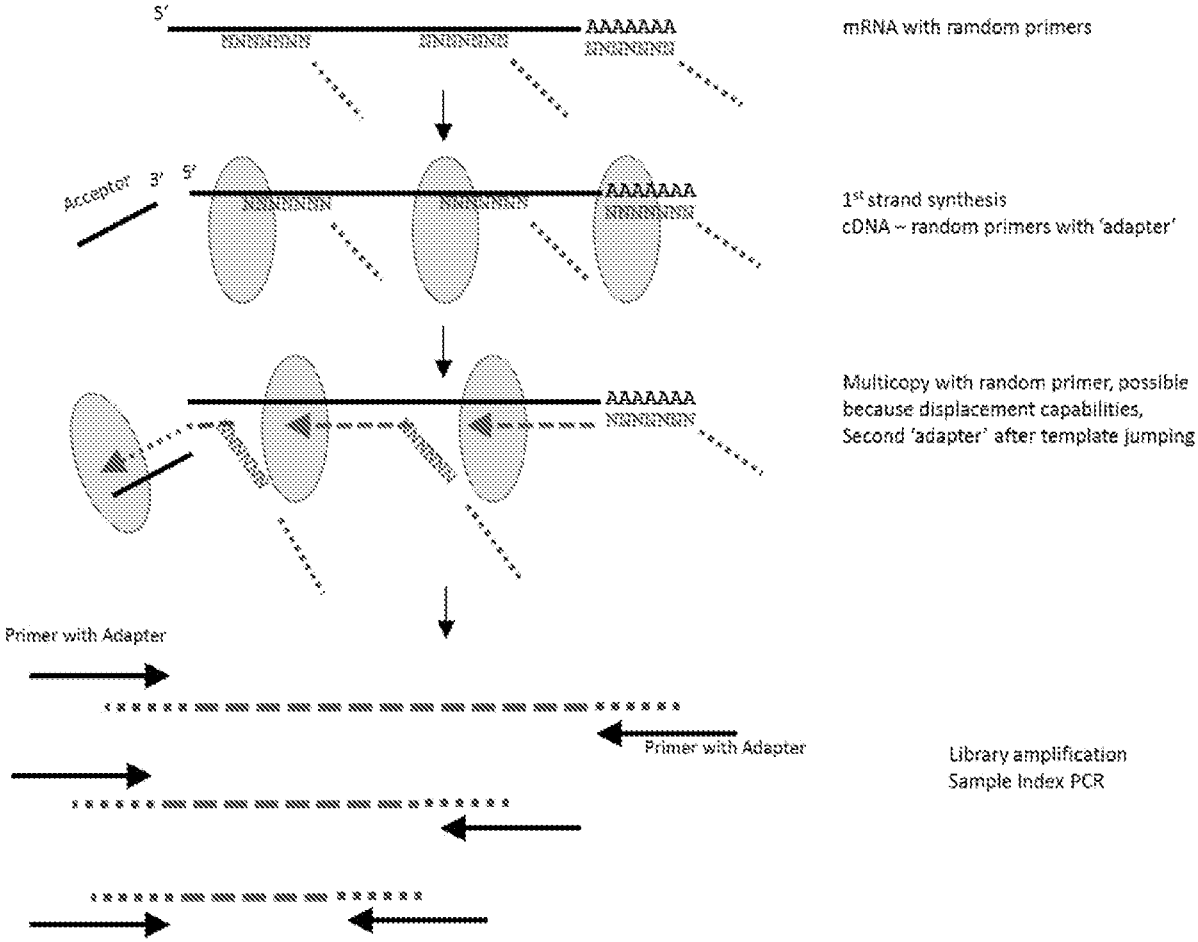


FIGURE 4

Example of a workflow using random primers for constructing a library

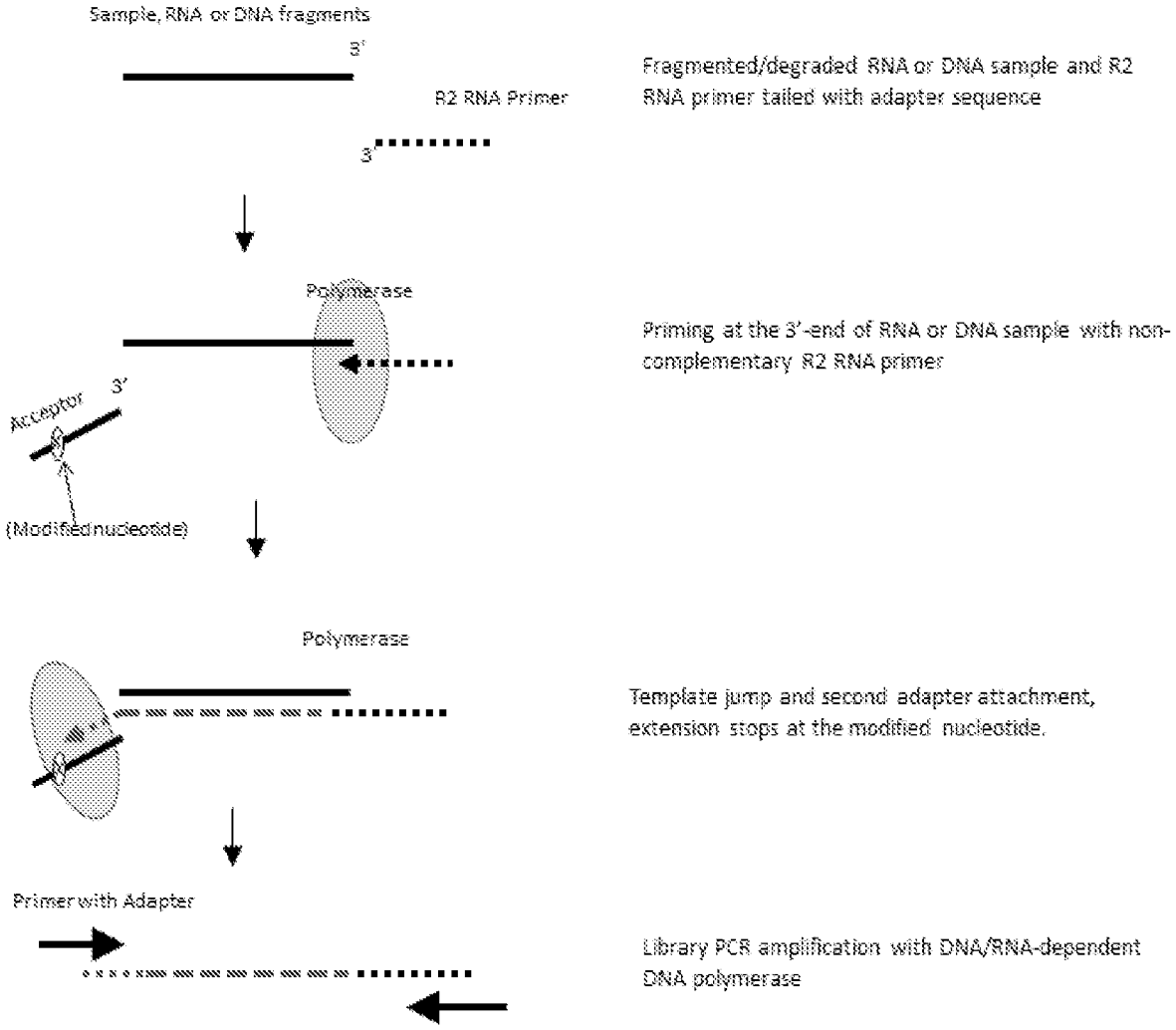


FIGURE 5A

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

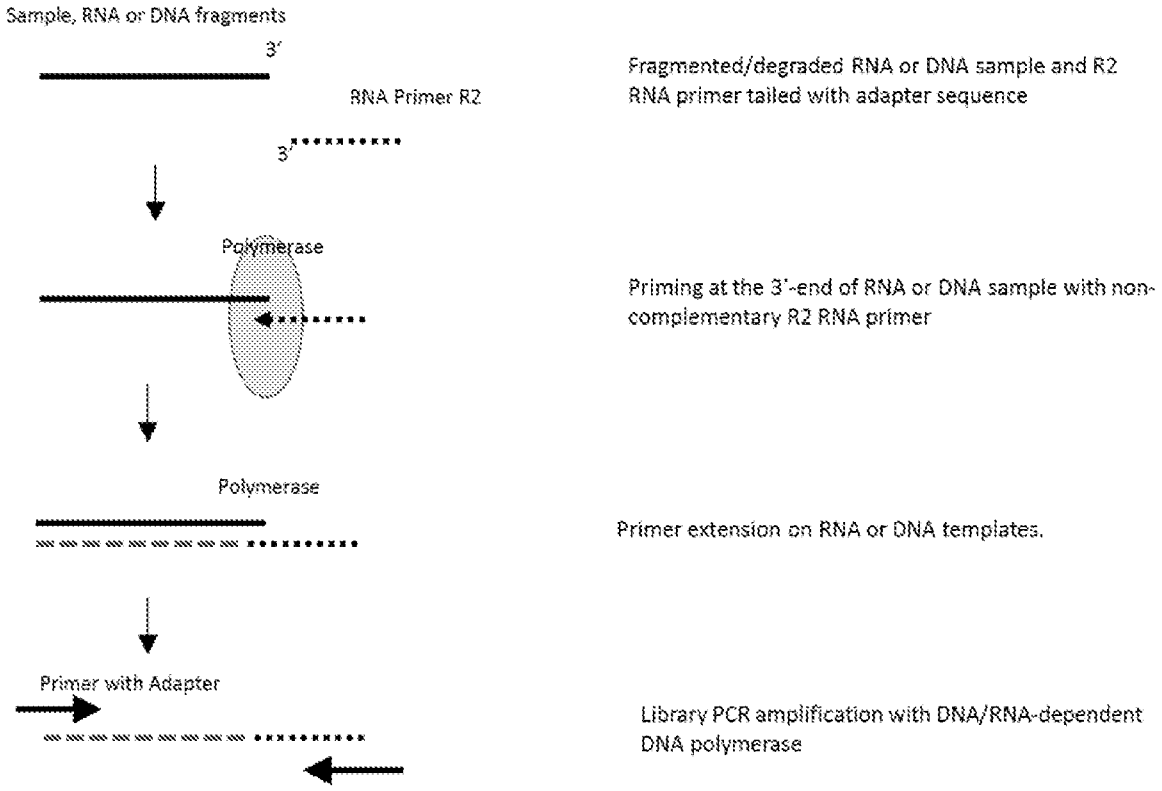


FIGURE 5B

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

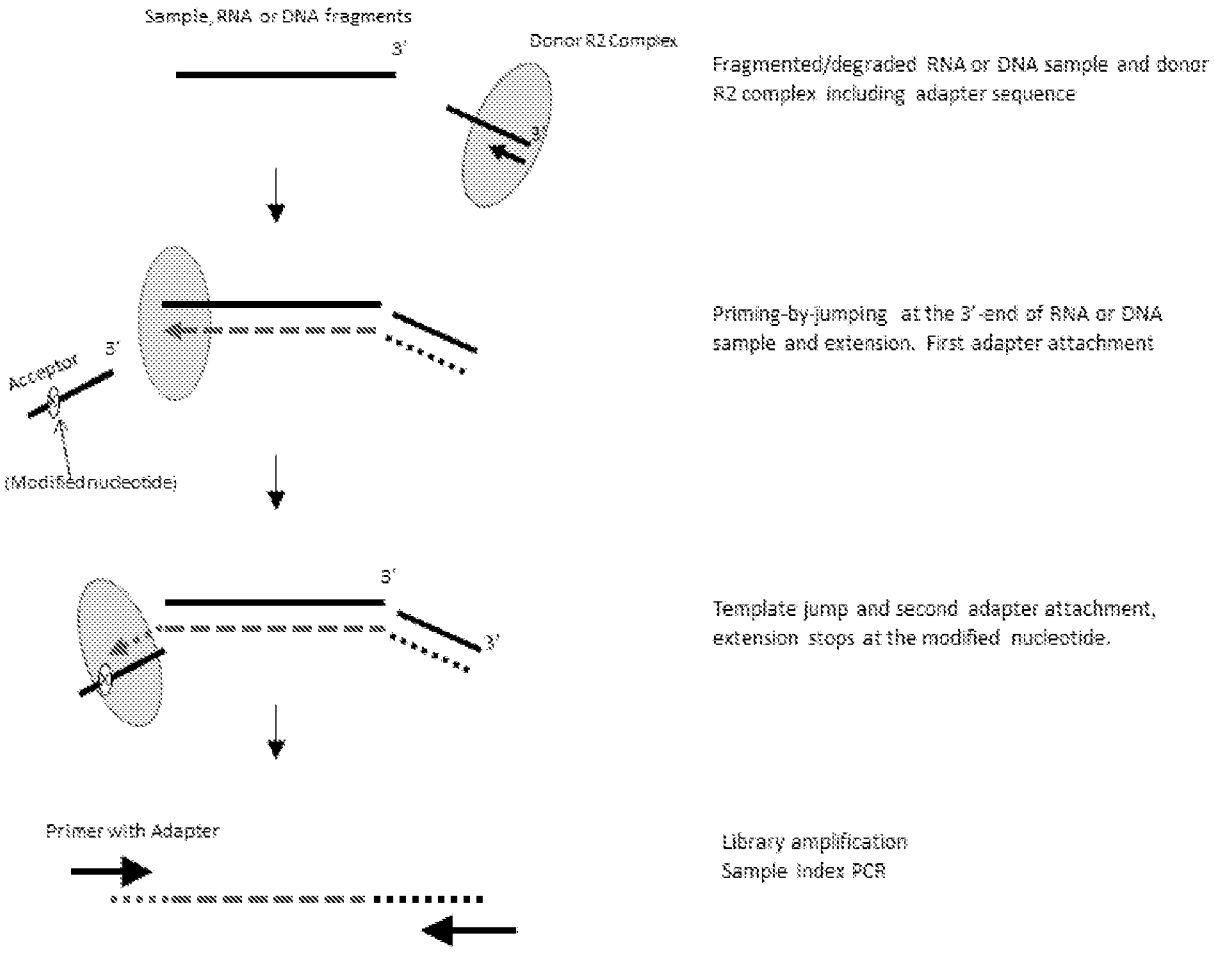


FIGURE 6A

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

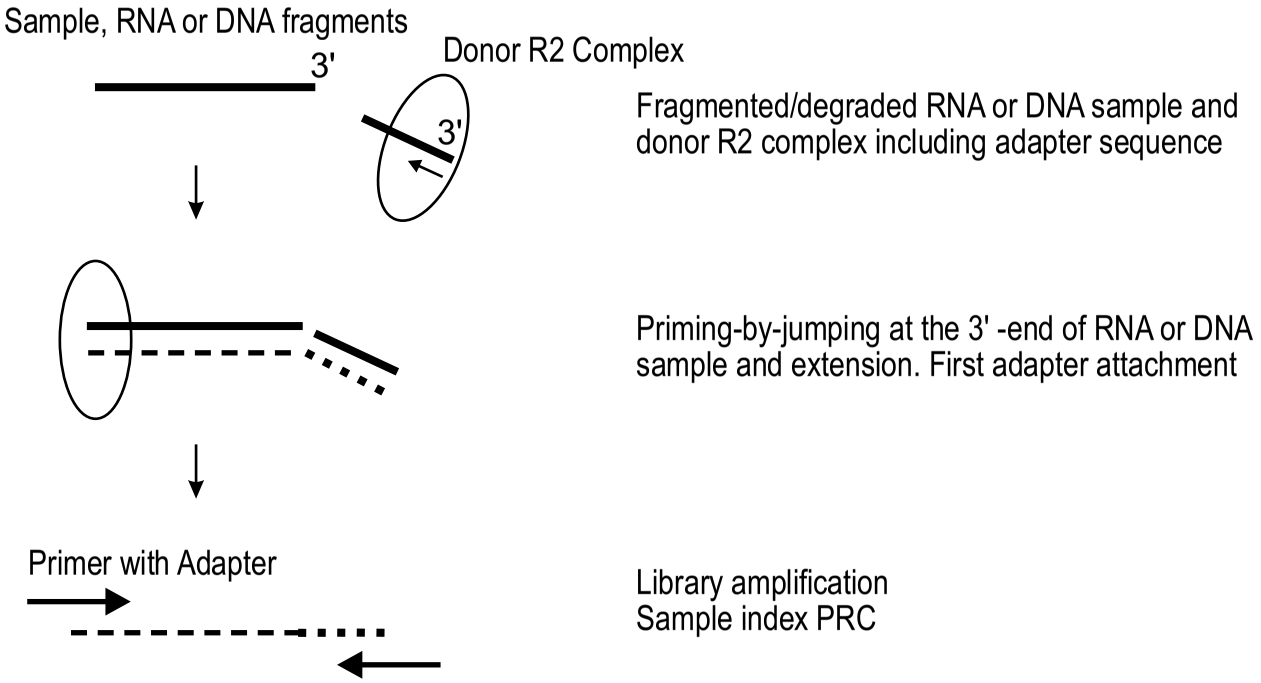


FIG. 6B

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

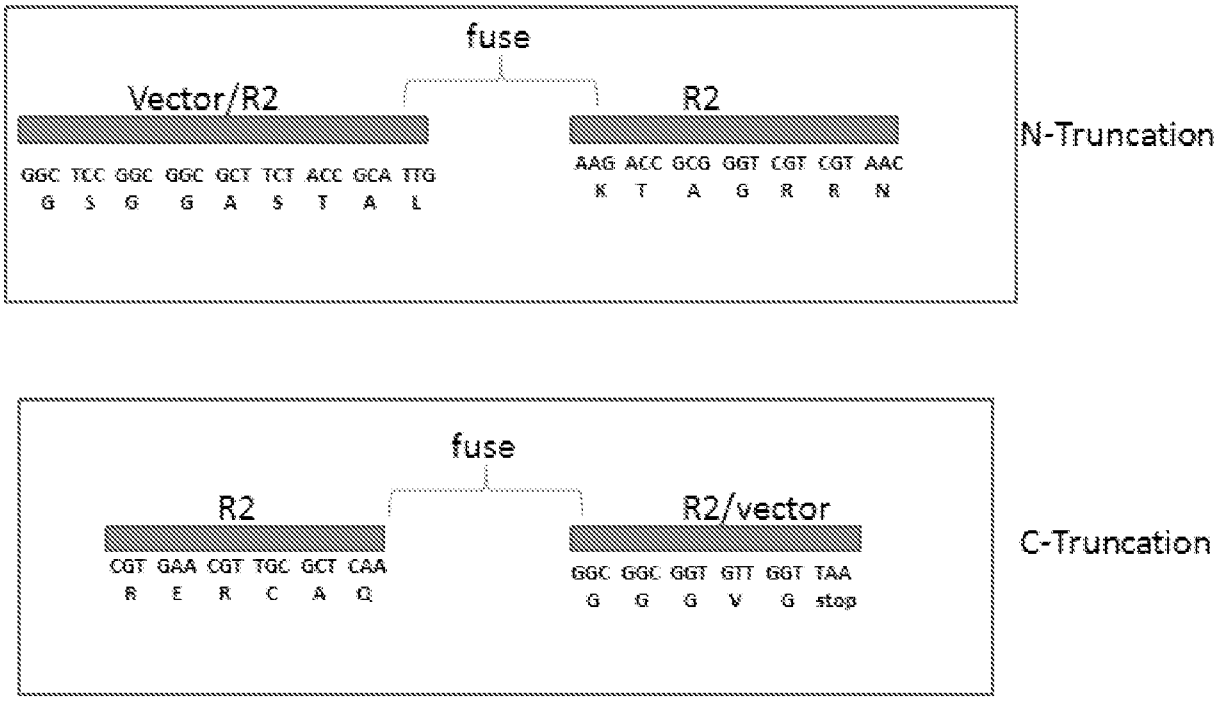
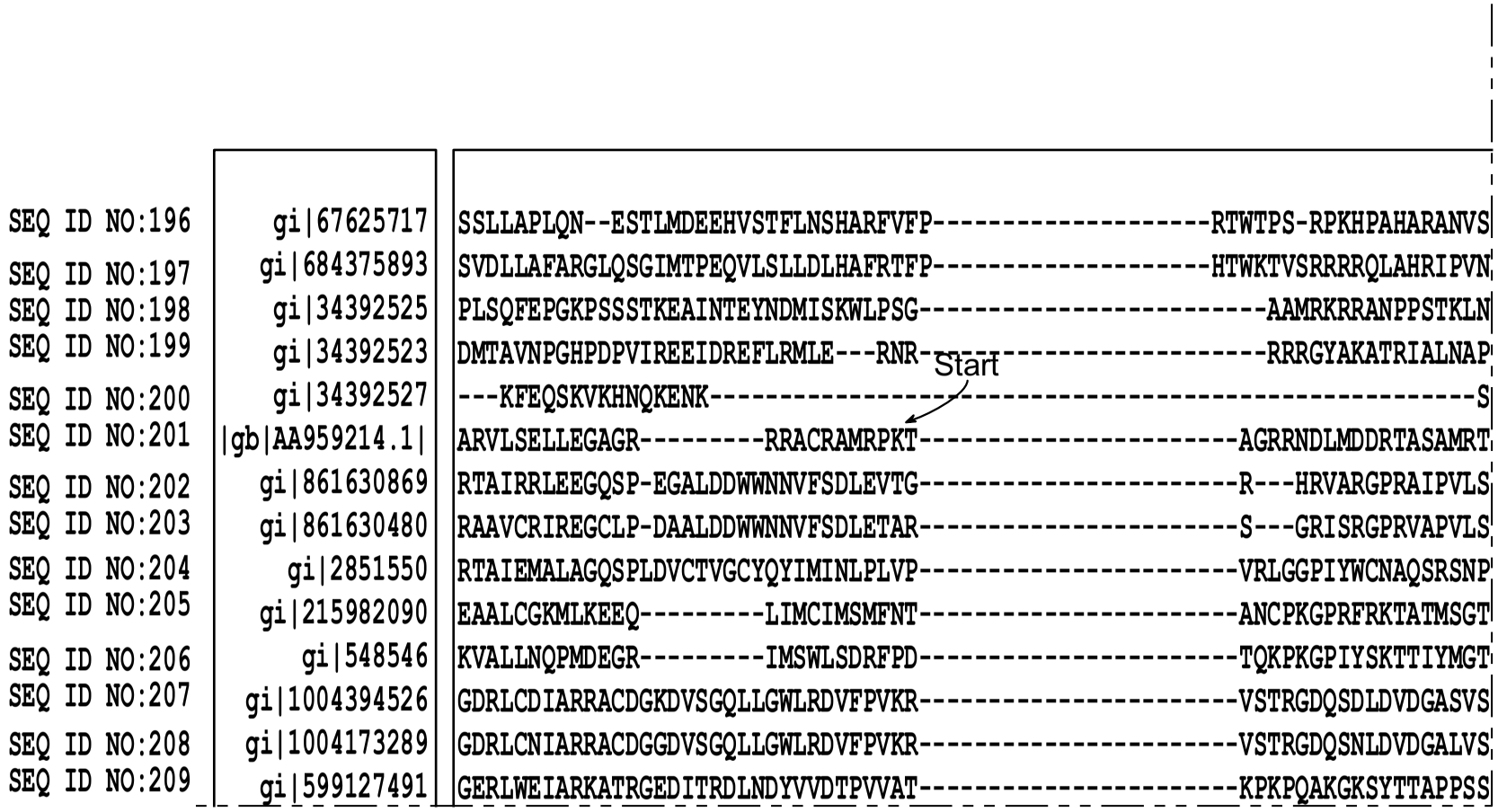


FIGURE 7

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

FIG. 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)

FIG. 8(CONT.)


Region - 1
RKKRRKIEYAMIQRLPMMRPKDASNTVIDGRWRNPYVANHSMIPDFDCFWTTVFTKTNSPDSREITPIIPMTPS-----
RKQIRRANPAAIQTLPMQRRKDSASAVLDGSWRDLYKGNCGLPTNAERPWKQVLSAPKRAGSRPSRIVVPSDWS-----
ARATRRLQYSRIQNLYKLNRSACAQEVLSGAWKVQS-GELNLKE-VQPFWEKMFRKESAKDRRKPKPTGEVLWG-----
GKVRRRAEYAAMQRQWKTKRGLCAREALEGTWKIPA-RTVSLSD-QEAFWRPLMESQSKNDLREPAKVGETLWG-----
KRQIRRAQYARTQINYKRNRQQAINSILSKQWRELDDRGPPLEM-VLPFWQKLLEKSSEHDTRQPEPVSGPIME-----
SRQKRRAEYARVQELYKKCRSRAAAEVIDGACGGVGMSLEEME----TYWRPILERVSDAPGPTPEALHALGRAEWHGC
KRKARRIEYRRMQQLWRTNMTKAAHKVLDGDAGSLPHPTLAAQ----LGFWKPVLEAESVDLAWPFAVGMP--------
KRRARRVEYRRMQQLWKVNMTKAAHKVLDGDTDSLPHPTLAEQ----LDFWRPVLEASSASFVRPVGAGKV--------
GETQRRQRIKESNNSWKKNMSKAAHIVLDGDTDACPAGLEGTE----ASGAIMRAGCPTTRHLRSRMQG----------
NKQQRQQRYARVQKLYKMNRKVAAKMVLE-ETDKIQIKLPDHD----PMFKFWESEFKEGEGMPERMPKDLKE------
NKQKRKQRYALVQSLYKKDISAAARVVLD-ENDKIATKIPPVR----HMFDYWKDVFATGGGSAATNINRAPP------
RRTARRREYARVQELYRKDPKACLARILGDRREGANRAPNRDP----APIDFWRGVFSEASAEVEGWAEEVSDHG----
RHTARTREYARVQELYRKDPKACLARIFGDRREGANRAPHRDP----AFIDFWRGVFSEASAEVEGCAEEVSDHG----
RRKRRRRLYGRMQEMMRRKPADCAKIVLDGERVASKINSEEFF----DYWEKLTTKEPSNWPLSSDTAQRE--------

FIG. 8(CONT.)

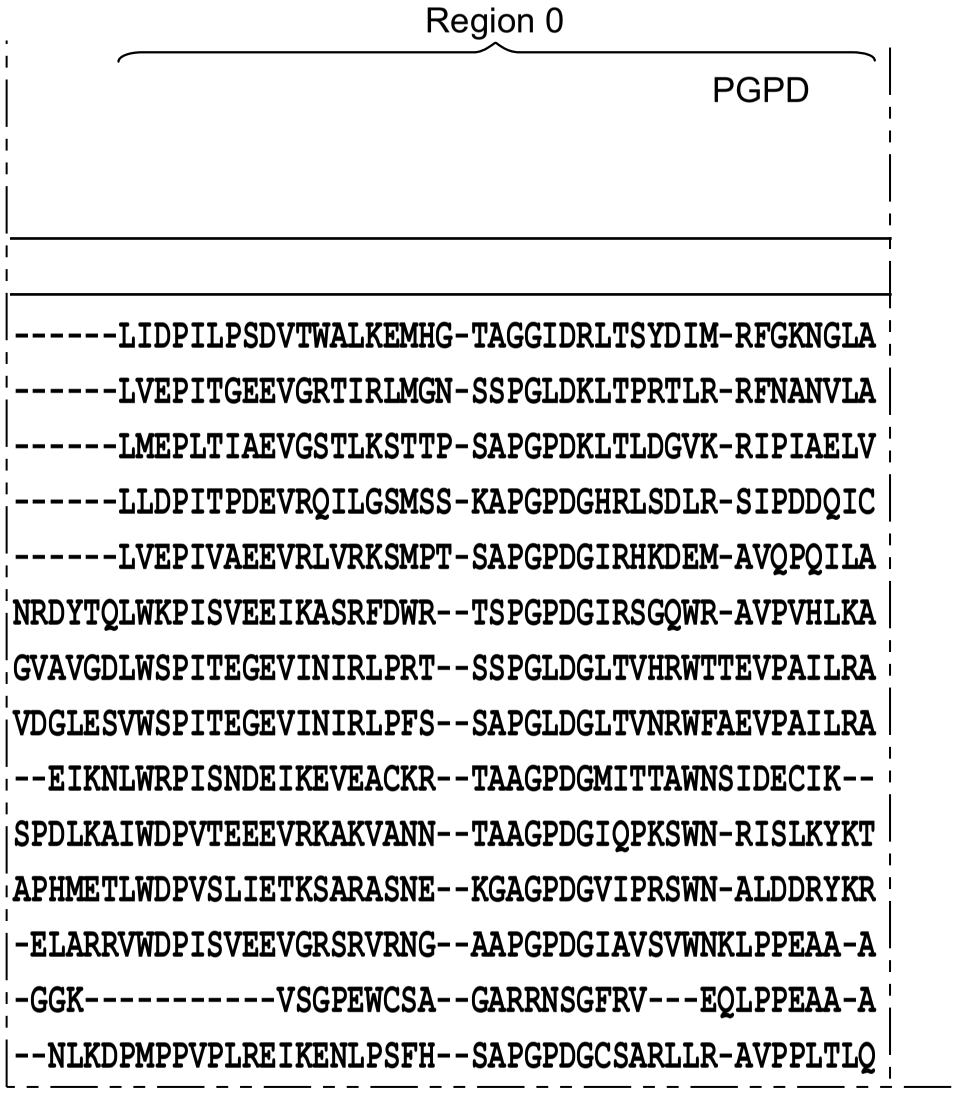

Region 0
PGPD
------LIDPILPSDVTWALKEMHG-TAGGIDRLTSYDIM-RFGKNGLA
------LVEPITGEEVGRTIRLMGN-SSPGLDKLTPRTLR-RFNANVLA
------LMEPLTIAEVGSTLKSTTP-SAPGPDKLTLDGVK-RIPIAELV
------LLDPITPDEVRQILGSMSS-KAPGPDGHRLSDLR-SIPDDQIC
------LVEPIVAEEVRLVRKSMPT-SAPGPDGIRHKDEM-AVQPQILA
NRDYTQLWKPISVEEIKASRFDWR--TSPGPDGIRSGQWR-AVPVHLKA
GVAVGDLWSPITEGEVINIRLPRT--SSPGLDGLTVHRWTTEVPAILRA
VDGLESVWSPITEGEVINIRLPFS--SAPGLDGLTVNRWFAEVPAILRA
--EIKNLWRPISNDEIKEVEACKR--TAAGPDGMITTAWNSIDECIK--
SPDLKAIWDPVTEEEVRKAKVANN--TAAGPDGIQPKSWN-RISLKYKT
APHMETLWDPVSLIETKSARASNE--KGAGPDGVIPRSWN-ALDDRYKR
-ELARRVWDPISVEEVGRSRVRNG--AAPGPDGIAVSVWNKLPPEAA-A
-GGK----------VSGPEWCSA--GARRNSGFRV---EQLPPEAA-A
--NLKDPMPPVPLREIKENLPSFH--SAPGPDGCSARLLR-AVPPLTLQ

FIG. 8(CONT.)

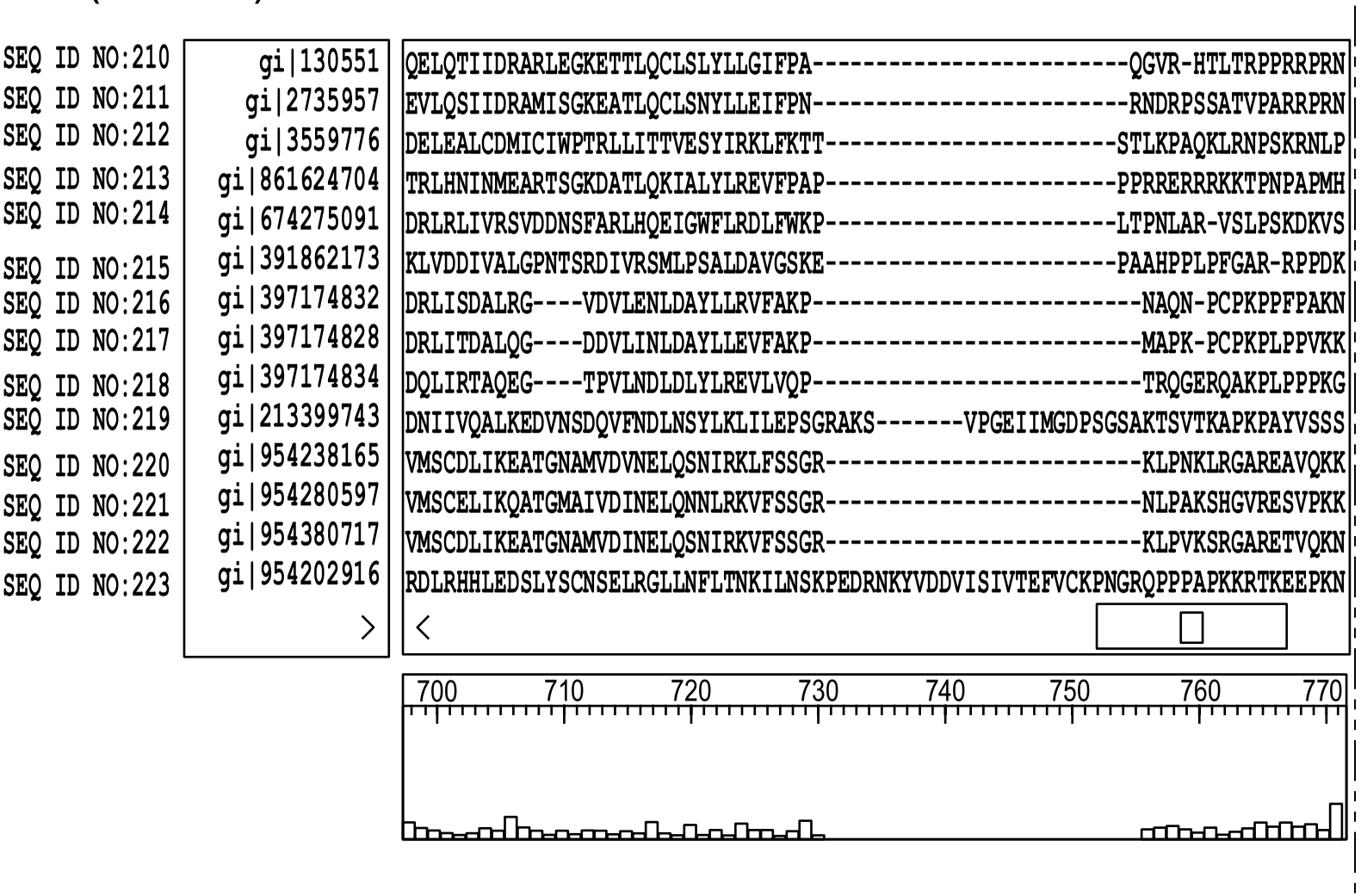

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SEQ ID NO:211
SEQ ID NO:212
SEQ ID NO:213
SEQ ID NO:214
SEQ ID NO:215
SEQ ID NO:216
SEQ ID NO:217
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SEQ ID NO:219
SEQ ID NO:220
SEQ ID NO:221
SEQ ID NO:222
SEQ ID NO:223
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gi|2735957
gi|3559776
gi|861624704
gi|674275091
gi|391862173
gi|397174832
gi|397174828
gi|397174834
gi|213399743
gi|954238165
gi|954280597
gi|954380717
gi|954202916
QELQTIIDRARLEGKETTLQCLSLYLLGIFPA----------QGVR-HTLTRPPRRPRN
EVLQSIIDRAMISGKEATLQCLSNYLLEIFPN----------RNDRPSSATVPARRPRN
DELEALCDMICIWPTRLLITTVESYIRKLFKTT----------STLKPAQKLRNPSKRNLP
TRLHNINMEARTSGKDATLQKIALYLREVFPAP----------PPRRERRRKKTPNPAPMH
DRLRLIVRSVDDNSFARLHQEIGWFLRDLFWKP----------LTPNLAR-VSLPSKDKVS
KLVDDIVALGPNTSRDIVRSMLPSALDAVGSKE----------PAAHPPLPFGAR-RPPDK
DRLISDALRG----VDVLENLDAYLLRVFAKP----------NAQN-PCPKPPFPAKN
DRLITDALQG----DDVLINLDAYLLEVFAKP----------MAPK-PCPKPLPPVKK
DQLIRTAQEG----TPVLNDLDLYLREVLVQP----------TRQGERQAKPLPPPKG
DNIIVQALKEDVNSDQVFNDLNSYLKLILEPSGRAKS-------VPGEIIMGDPSGSAKTSVTKAPKPAYVSSS
VMSCDLIKEATGNAMVDVNELQSNIRKLFSSGR----------KLPNKLRGAREAVQKK
VMSCELIKQATGMAIVDINELQNNLRKVFSSGR----------NLPAKSHGVRESVPKK
VMSCDLIKEATGNAMVDINELQSNIRKVFSSGR----------KLPVKSRGARETVQKN
RDLRHHLEDSLYSCNSELRGLLNFLTNKILNSKPEDRNKYVDDVISIVTEFVCKPNGRQPPPAPKKRTKEEPKN
700
710
720
730
740
750
760
770

FIG. 8(CONT.)

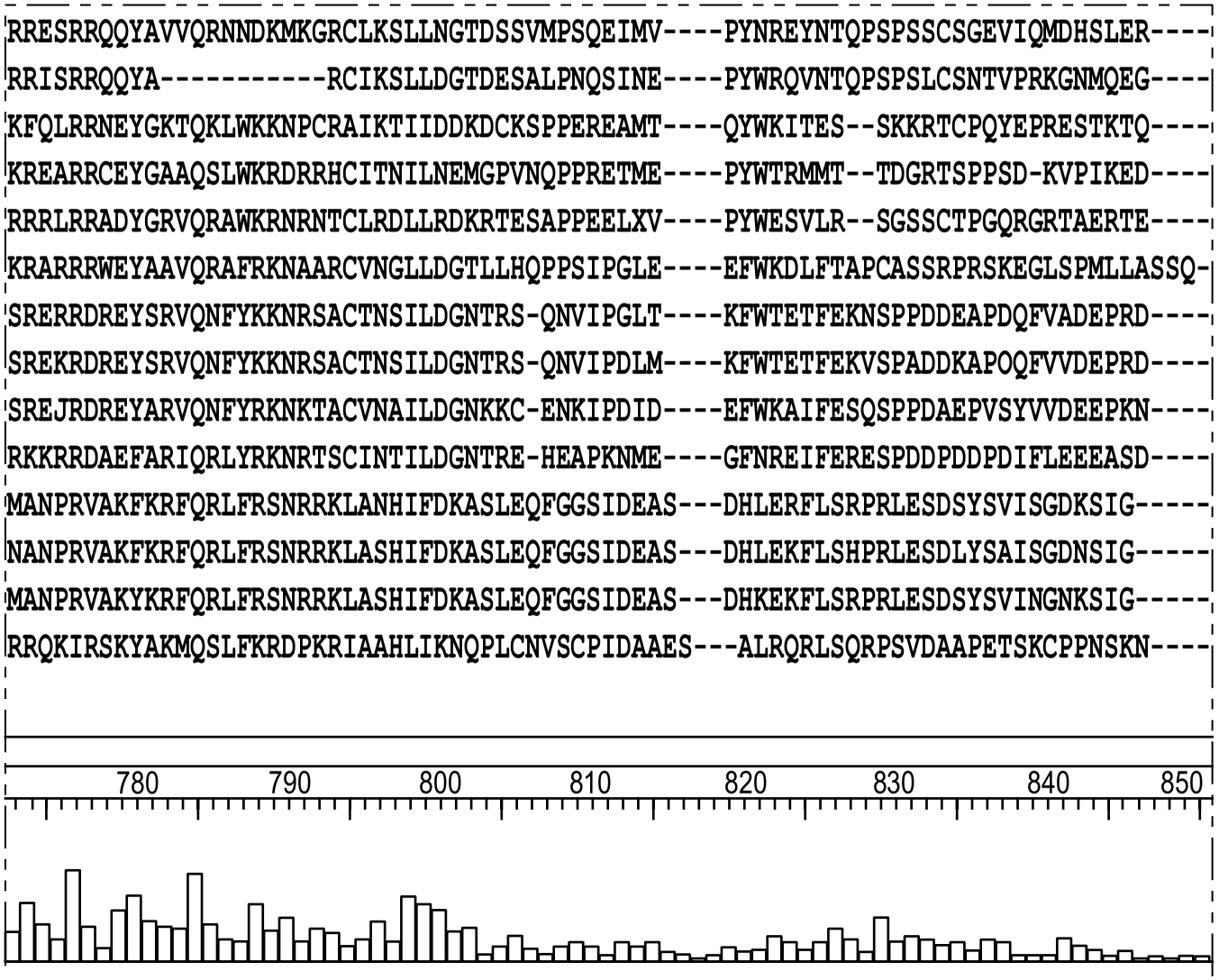

RRESRRQQYAVVQRNNDKMKGRCLKSLLNGTDSSVMPSQEIMV----PYNREYNTQPSPSSCSGEVIQMDHSLER----
RRISRRQQYA----------RCIKSLLDGTDESALPNQSINE----PYWRQVNTQPSPSLCSNTVPRKGNMQEG----
KFQLRRNEYGKTQKLWKKNPCRAIKTIIDDKDCKSPPEREAMT----QYWKITES--SKKRTCPQYEPRESTKTQ----
KREARRCEYGAAQSLWKRDRRHCITNILNEMGPVNQPPRETME----PYWTRMMT--TDGRTSPPSD-KVPIKED----
RRRLRRADYGRVQRAWKRNRNTCLRDLLRDKRTESAPPEELXV----PYWESVLR--SGSSCTPGQRGRTAERTE----
KRARRRWEYAAVQRAFRKNAARCVNGLLDGTLLHQPPSIPGLE----EFWKDLFTAPCASSRPRSKEGLSPMLLASSQ-
SRERRDREYSRVQNFYKKNRSACTNSILDGNTRS-QNVIPGLT----KFWTETFEKNSPPDDEAPDQFVADEPRD----
SREKRDREYSRVQNFYKKNRSACTNSILDGNTRS-QNVIPDLM----KFWTETFEKVSPADDKAPOQFVVDEPRD----
SREJRDREYARVQNFYRKNKTACVNAILDGNKKC-ENKIPDID----EFWKAIFESQSPPDAEPVSYVVDEEPKN----
RKKRRDAEFARIQRLYRKNRTSCINTILDGNTRE-HEAPKNME----GFNREIFERESPDDPDDPDIFLEEEASD----
MANPRVAKFKRFQRLFRSNRRKLANHIFDKASLEQFGGSIDEAS---DHLERFLSRPRLESDSYSVISGDKSIG-----
NANPRVAKFKRFQRLFRSNRRKLASHIFDKASLEQFGGSIDEAS---DHLEKFLSHPRLESDLYSAISGDNSIG-----
MANPRVAKYKRFQRLFRSNRRKLASHIFDKASLEQFGGSIDEAS---DHKEKFLSRPRLESDSYSVINGNKSIG-----
RRQKIRSKYAKMQSLFKRDPKRIAAHLIKNQPLCNVSCPIDAAES---ALRQRLSQRPSVDAAPETSKCPPNSKN----
780
790
800
810
820
830
840
850

FIG. 8(CONT.)

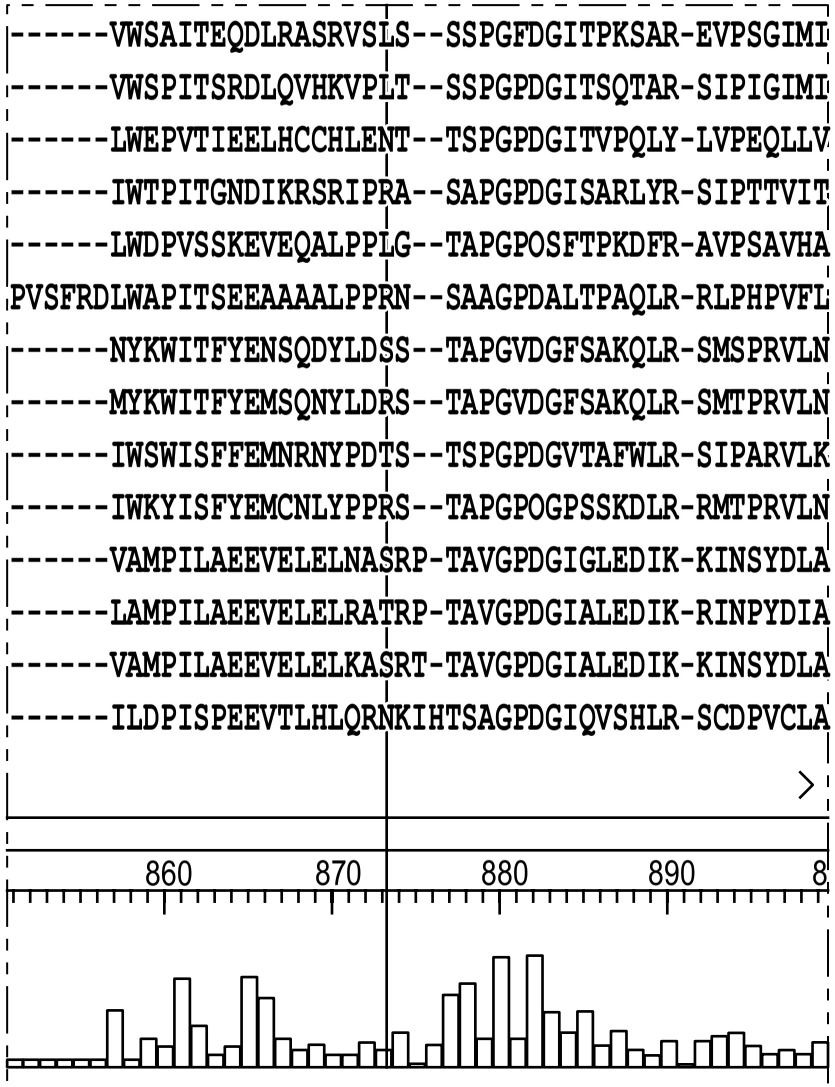

------VWSAITEQDLRASRVSLS--SSPGFDGITPKSAR-EVPSGIMI
------VWSPITSRDLQVHKVPLT--SSPGPDGITSQTAR-SIPIGIMI
------LWEPVTIEELHCCHLENT--TSPGPDGITVPQLY-LVPEQLLV
------IWTPITGNDIKRSRIPRA--SAPGPDGISARLYR-SIPTTVIT
------LWDPVSSKEVEQALPPLG--TAPGPOSFTPKDFR-AVPSAVHA
PVSFRDLWAPITSEEAAAALPPRN--SAAGPDALTPAQLR-RLPHPVFL
------NYKWITFYENSQDYLDSS--TAPGVDGFSAKQLR-SMSPRVLN
------MYKWITFYEMSQNYLDRS--TAPGVDGFSAKQLR-SMTPRVLN
------IWSWISFFEMNRNYPDTS--TSPGPDGVTAFWLR-SIPARVLK
------IWKYISFYEMCNLYPPRS--TAPGPOGPSSKDLR-RMTPRVLN
------VAMPILAEEVELELNASRP-TAVGPDGIGLEDIK-KINSYDLA
------LAMPILAEEVELELRATRP-TAVGPDGIALEDIK-RINPYDIA
------VAMPILAEEVELELKASRT-TAVGPDGIALEDIK-KINSYDLA
------ILDPISPEEVTLHLQRNKIHTSAGPDGIQVSHLR-SCDPVCLA
860
870
880
890
8

FIG. 9


GGLA
SEQ ID NO:224 gi|67625717 TEISCAPLKRQQREKILKDNLLPRLLYPLTLGIVELKTLKSHDRNIETAIRKWLR-LPSDTPLAYFHSPVAAGGLGILHLSSSVPFHRRKRLE
SEQ ID NO:225 gi|684375893 EEVTRAPLKPHQREDTTRKULIPRLTYSLVLGQVERNTLKRLDNYIRQYIRGWLR-LPKDTPISYIHAGKQEGGLGIPSLSATIPMQRKTRHE
SEQ ID NO:226 gi|34392525 AELSRAPLKPQQRLYLLRVELLPKLQHGLVLSSCAKRALTYLDKSVRSAIRRWLT-LPKDTPTAFYMAKACDGGLGITRLENTIPILKRNRMM
SEQ ID NO:227 gi|34392523 ANLSRAPLKPQQRLYILCTNLLPSIYMQLVLSSTSKKFLKYLDRCVRVAVRRWLR-LPKDTPKAYFHAKCNDGGLGVPELQRVIPLQKAGRNL
SEQ ID NO:228 gi|34392527 KETTEAPLKPQQRLFILRNYILPRSIMILTPINTTARLLNQLDSAIRINVRRWLK-LPKDTPLGYLYSDYKDGGLGVPRLLSRVPLLTIRKHA
SEQ ID NO:229 |gb|AA959214.1| NNISRAPLKPQQRLEILRAHLIPRFQHGFVLGNISDDRLRELDVQIRKAVGQWLR-LPADVPKAYYMAAVQDGGLAIPSVRATIPDLIVRKFG
SEQ ID NO:230 gi|861630869 ERITRAPLKPQQRLKILRTYLIPRYRHNLVLGRVSYSHLRKLDMQTRAAVRRWLV-LPDDVPVAFFHCPINQGGLGIQSFETAIPRLTLLKLN
SEQ ID NO:231 gi|861630480 DRITRAPLKPQQRLKILKTFLIPRFIHILVLGRTSYGLLRKLDRQIRAAVRRWLR-LEPDIPKAFFHSPIASGGLGILSYETAIPRLVLAKLD
SEQ ID NO:232 gi|2851550 QRVTAAPLKPQQRNAILGHFLIPRFIHKLVLGRTSNADVRFGDKIIRKTVRGWLR-LPHDTPIGYFHAPIKEGGLGIPARESRIPELLKSRIE
SEQ ID NO:233 gi|215982090 ERLTKAPLKPQQRIHMLNAYVIPKYQDKLVLSKTTAKGLKRTDRQIRQYVRRWLK-LPMDVPIAYLMAPVKSGGLNIPCLQYWIPLLRVNRVN
SEQ ID NO:234 gi|548646 SKLTKGPLKPQQRIHLLKTFVIPKELNRLVLSRTTATGLCKMDLLIRKYVRRWLR-LPGDVPVAFLYAPVKAGGKGIFCLKQWIPLMRFLRLN
SEQ ID NO:235 gi|1004394526 KRITCAPLKPQQRHHLLRVFFLPKFYHANTFGRLNAGVLRRLDVVVRTYVRTWLR-FPHDIPVGYFHAPTKSGGLGIPQLSRFIPFLRLKRFD
SEQ ID NO:236 gi|1004173289 KRNTCAPLKPQQRMMLLRVFFLPKFYMANTFGRLNAGVLRRLNVVVRTSVRTWLR-LPHDIPVGKFMAPYKSGGLGIFQLSRLIPFLRLKRFD
SEQ ID NO:237 gi|599127491 DRISSAPLKPQQRLYILRGFLVPRLIYRLVLGRWTAGTLLKLDRQIRAAIRRWIG-LPHDCPLGYFKAKIGSGGLGTPSMRIMIPELLIRRLT
SEQ ID NO:238 gi|130551 QRLTKAPLKPQQRLFALRTVLIPQLYHKLALGSVAIGVLRKTDKLIRYYVRRWLN-LPLDVPIAFVHAPPKSGGLGIFSLRWVAPMLRLRRLS
SEQ ID NO:239 gi|2735957 ERLMQSPLKPQQKLFALRTVLVPQLYHKLTLGSVALGVLRKCDKLVRSFARKLLG-LPLDVSVAFYMAPHSCGGLGIFSVRWIAPMLRTKRLA

FIG. 9

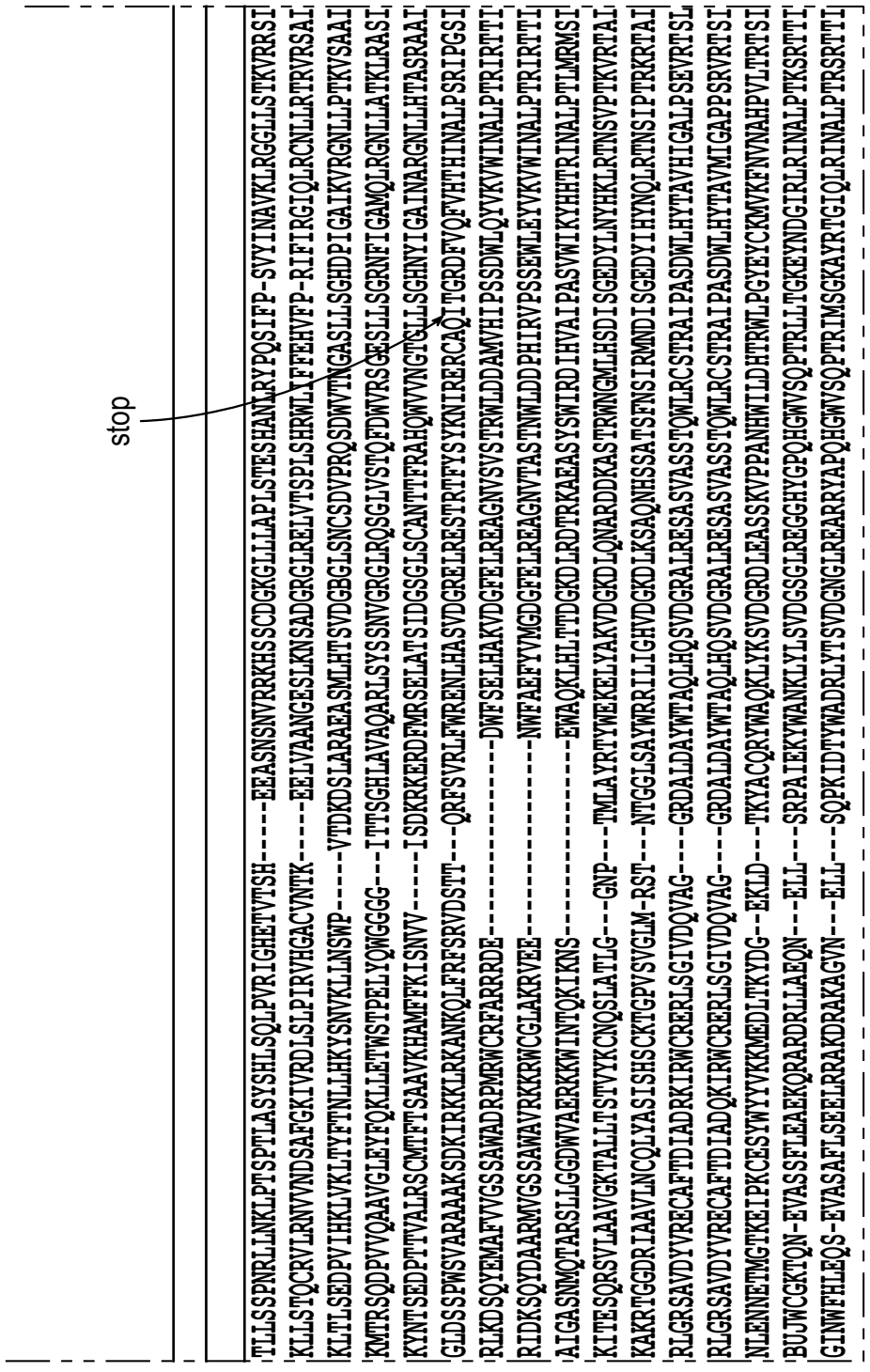


FIG. 9 (CONT.)

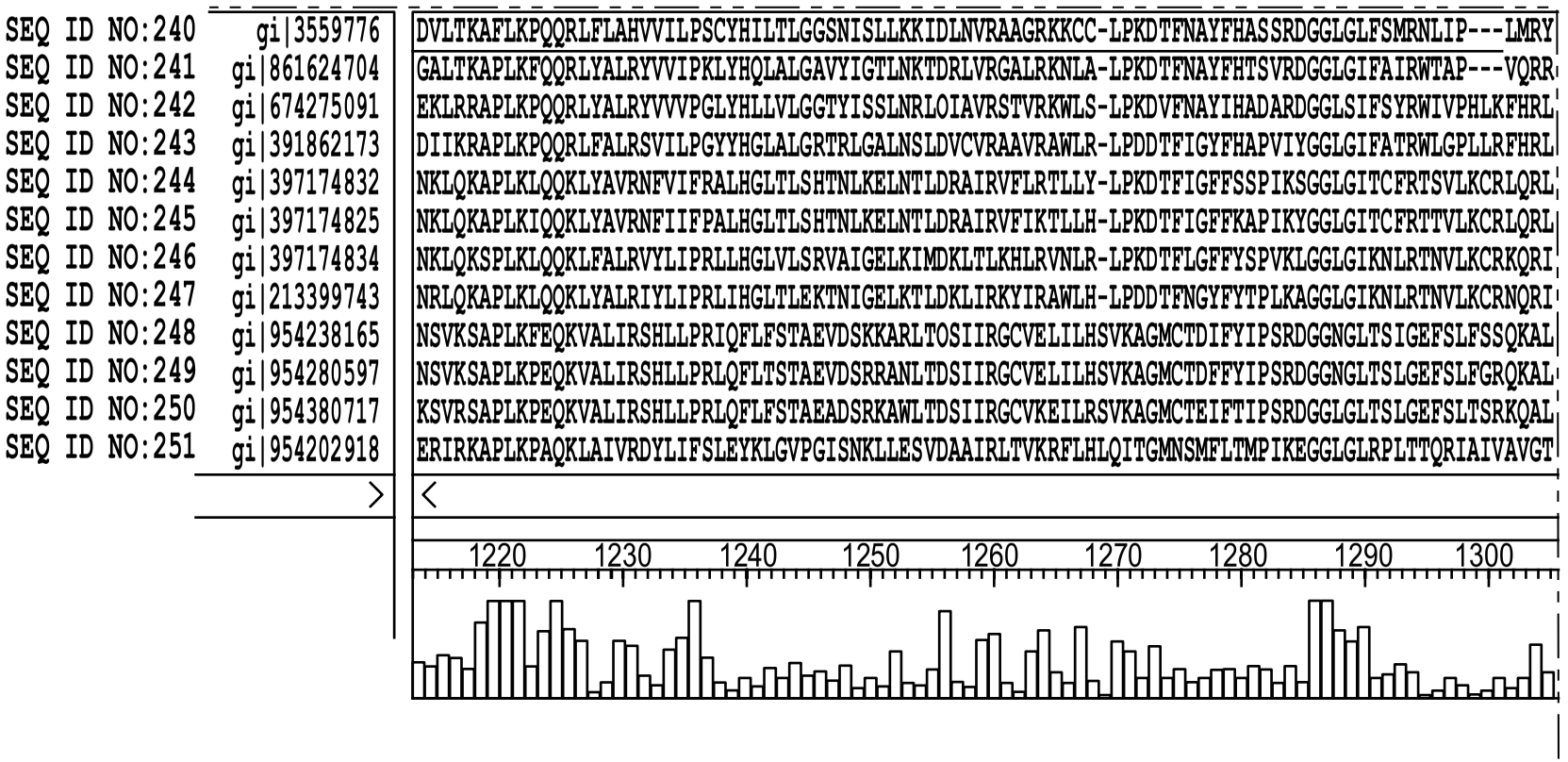
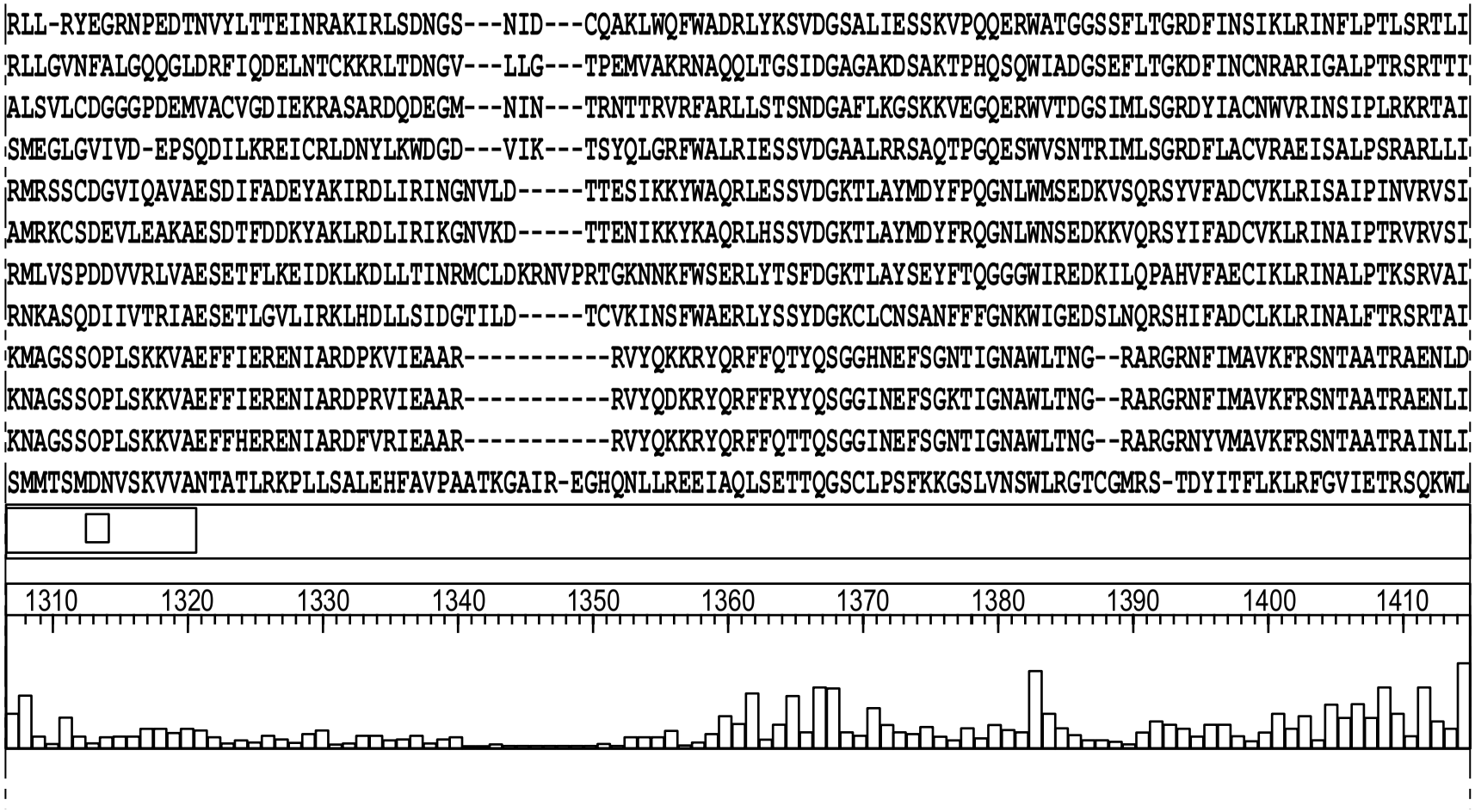

SEQ ID NO:240 gi|3559776 DVLTKAFLKPQQRLFLAHVVILPSCYHILTLGGSNISLLKKIDLNVRAAGRKKCC-LPKDTFNAYFHASSRDGGLGLFSMRNLIP---LMRY
SEQ ID NO:241 gi|861624704 GALTKAPLKFQQRLYALRYVVIPKLYHQLALGAVYIGTLNKTDRLVRGALRKNLA-LPKDTFNAYFHTSVRDGGLGIFAIRWTAP---VQRR
SEQ ID NO:242 gi|674275091 EKLRRAPLKPQQRLYALRYVVVPGLYHLLVLGGTYISSLNRLQIAVRSTVRKWLS-LPKDVFNAYIHADARDGGLSIFSYRWIVPHLKFHRL
SEQ ID NO:243 gi|391862173 DIIKRAPLKPQQRLFALRSVILPGYYHGLALGRTRLGALNSLDVCVRAAVRAWLR-LPDDTFIGYFHAPVIYGGLGIFATRWLGPLLRFHRL
SEQ ID NO:244 gi|397174832 NKLQKAPLKLQQKLYAVRNFVIFRALHGLTLSHTNLKELNTLDRAIRVFLRTLLY-LPKDTFIGFFSSPIKSGGLGITCFRTSVLKCRLQRL
SEQ ID NO:245 gi|397174825 NKLQKAPLKIQQKLYAVRNFIIFPALHGLTLSHTNLKELNTLDRAIRVFIKTLLH-LPKDTFIGFFKAPIKYGGLGITCFRTTVLKCRLQRL
SEQ ID NO:246 gi|397174834 NKLQKSPLKLQQKLFALRVYLIPRLLHGLVLSRVAIGELKIMDKLTLKHLRVNLR-LPKDTFLGFFYSPVKLGGLGIKNLRTNVLKCRKQRI
SEQ ID NO:247 gi|213399743 NRLQKAPLKLQQKLYALRIYLIPRLIHGLTLEKTNIGELKTLDKLIRKYIRAWLH-LPDDTFNGYFYTPLKAGGLGIKNLRTNVLKCRNQRI
SEQ ID NO:248 gi|954238165 NSVKSAPLKFEQKVALIRSHLLPRIQFLFSTAEVDSKKARLTOSIIRGCVELILHSVKAGMCTDIFYIPSRDGGNGLTSIGEFSLFSSQKAL
SEQ ID NO:249 gi|954280597 NSVKSAPLKPEQKVALIRSHLLPRLQFLTSTAEVDSRRANLTDSIIRGCVELILHSVKAGMCTDFFYIPSRDGGNGLTSLGEFSLFGRQKAL
SEQ ID NO:250 gi|954380717 KSVRSAPLKPEQKVALIRSHLLPRLQFLFSTAEADSRKAWLTDSIIRGCVKEILRSVKAGMCTEIFTIPSRDGGLGLTSLGEFSLTSRKQAL
SEQ ID NO:251 gi|954202918 ERIRKAPLKPAQKLAIVRDYLIFSLEYKLGVPGISNKLLESVDAAIRLTVKRFLHLQITGMNSMFLTMPIKEGGLGLRPLTTQRIAIVAVGT
1220 1230 1240 1250 1260 1270 1280 1290 1300

FIG. 9(CONT.)


RLL-RYEGRNPEDTNVYLTTEINRAKIRLSDNGS---NID---CQAKLWQFWADRLYKSVDGSALIESSKVPQQERWATGGSSFLTGRDFINSIKLRINFLPTLSRTLI
RLLGVNFALGQQGLDRFIQDELNTCKKRLTDNGV---LLG---TPEMVAKRNAQQLTGSIDGAGAKDSAKTPHQSQWIADGSEFLTGKDFINCNRARIGALPTRSRTTI
ALSVLCDGGGPDEMVACVGDIEKRASARDQDEGM---NIN---TRNTTRVRFARLLSTSNDGAFLKGSKKVEGQERWVTDGSIMLSGRDYIACNWVRINSIPLRKRTAI
SMEGLGVIVD-EPSQDILKREICRLDNYLKWDGD---VIK---TSYQLGRFWALRIESSVDGAALRRSAQTPGQESWVSNTRIMLSGRDFLACVRAEISALPSRARLLI
RMRSSCDGVIQAVAESDIFADEYAKIRDLIRINGNVLD-----TTESIKKYWAQRLESSVDGKTLAYMDYFPQGNLWMSEDKVSQRSYVFADCVKLRISAIPINVRVSI
AMRKCSDEVLEAKAESDTFDDKYAKLRDLIRIKGNVKD-----TTENIKKYKAQRLHSSVDGKTLAYMDYFRQGNLWNSEDKKVQRSYIFADCVKLRINAIPTRVRVSI
RMLVSPDDVVRLVAESETFLKEIDKLKDLLTINRMCLDKRNVPRTGKNNKFWSERLYTSFDGKTLAYSEYFTQGGGWIREDKILQPAHVFAECIKLRINALPTKSRVAI
RNKASQDIIVTRIAESETLGVLIRKLHDLLSIDGTILD-----TCVKINSFWAERLYSSYDGKCLCNSANFFFGNKWIGEDSLNQRSHIFADCLKLRINALFTRSRTAI
KMAGSSOPLSKKVAEFFIERENIARDPKVIEAAR----------RVYQKKRYQRFFQTYQSGGHNEFSGNTIGNAWLTNG--RARGRNFIMAVKFRSNTAATRAENLD
KNAGSSOPLSKKVAEFFIERENIARDPRVIEAAR----------RVYQDKRYQRFFRYYQSGGINEFSGKTIGNAWLTNG--RARGRNFIMAVKFRSNTAATRAENLI
KNAGSSOPLSKKVAEFFHERENIARDFVRIEAAR----------RVYQKKRYQRFFQTTQSGGINEFSGNTIGNAWLTNG--RARGRNYVMAVKFRSNTAATRAINLI
SMMTSMDNVSKVVANTATLRKPLLSALEHFAVPAATKGAIR-EGHQNLLREEIAQLSETTQGSCLPSFKKGSLVNSWLRGTCGMRS-TDYITFLKLRFGVIETRSQKWL
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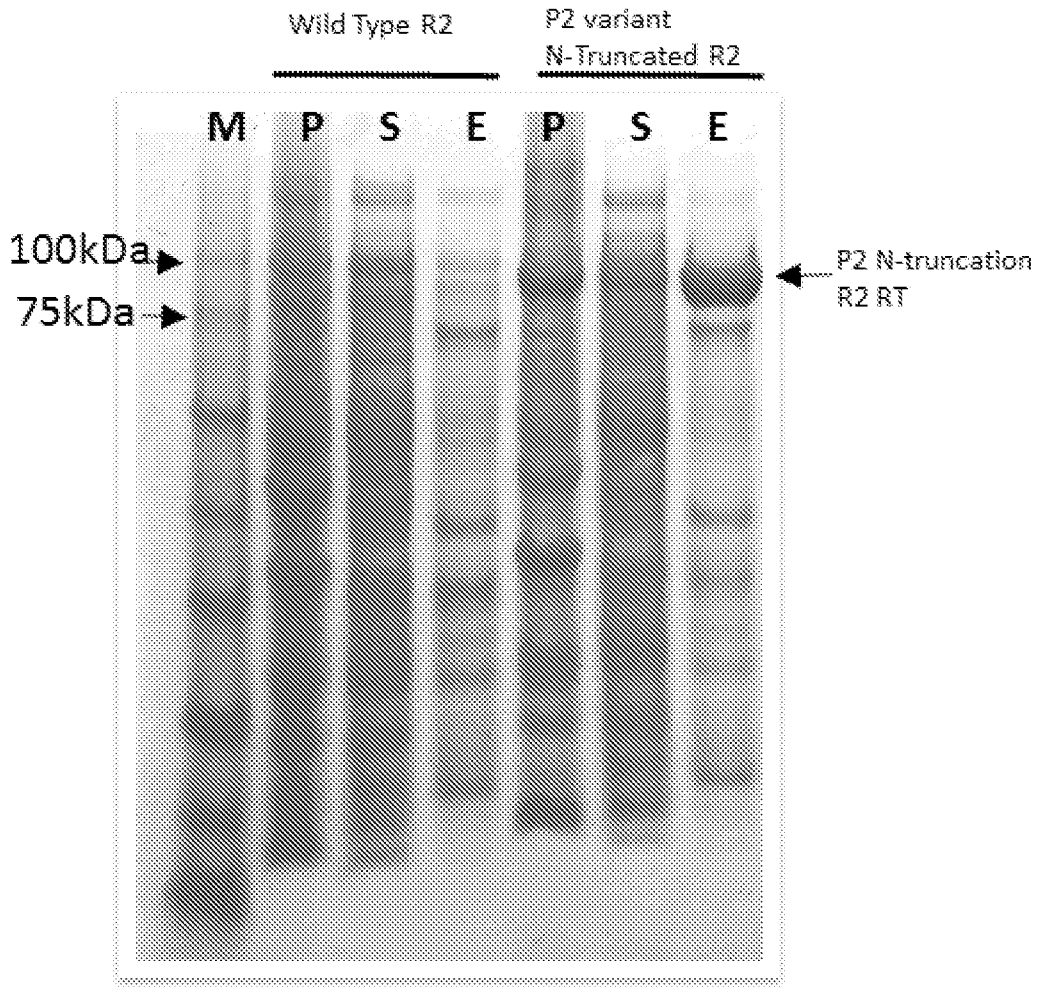


FIGURE 10

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

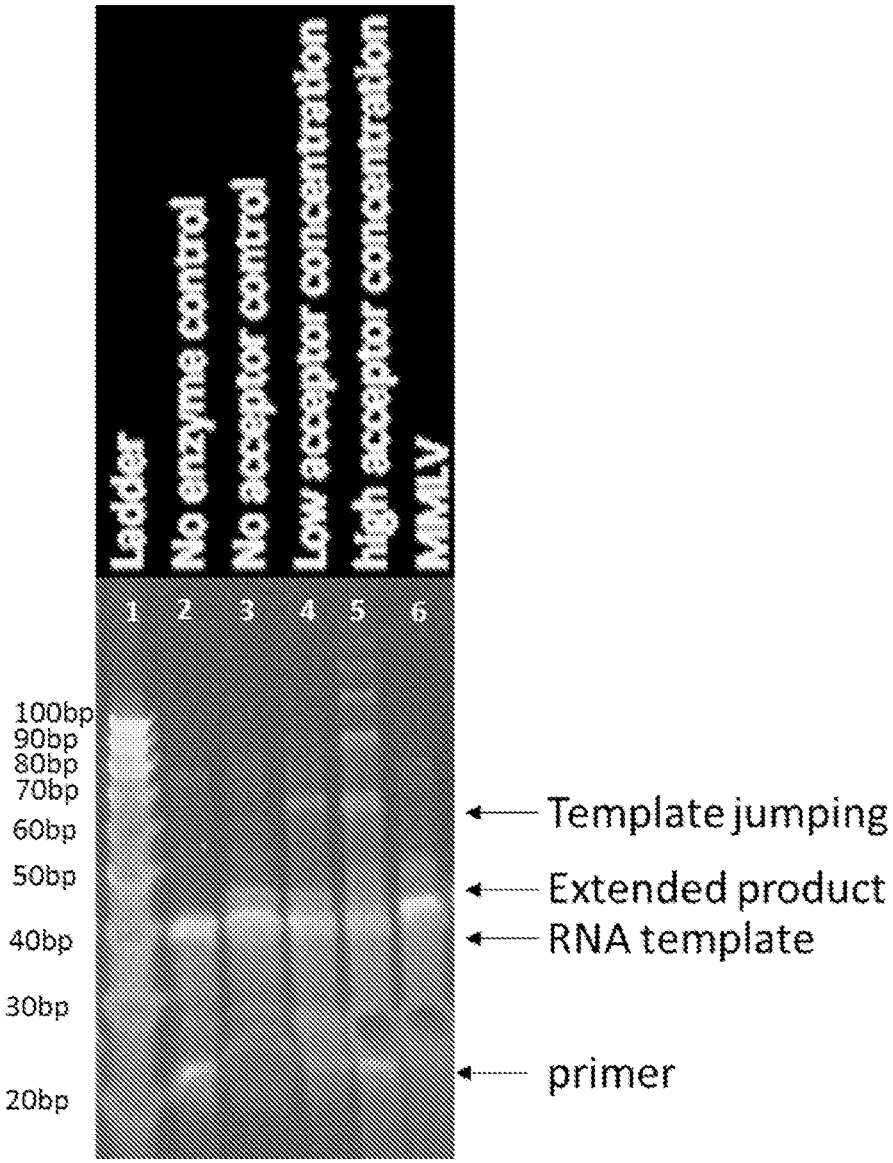


FIGURE 11

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

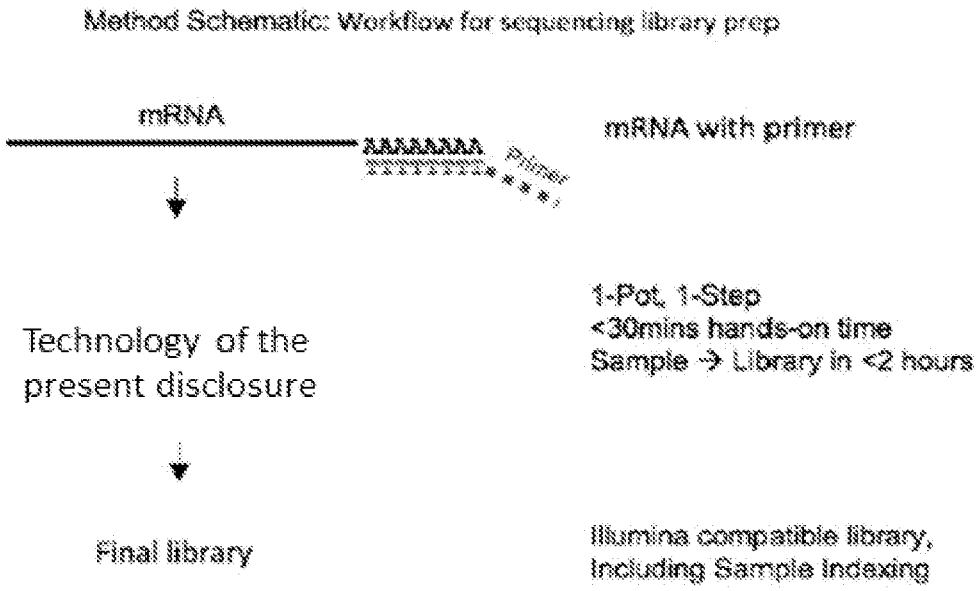


FIGURE 12A

Workflow for sequencing library preparation

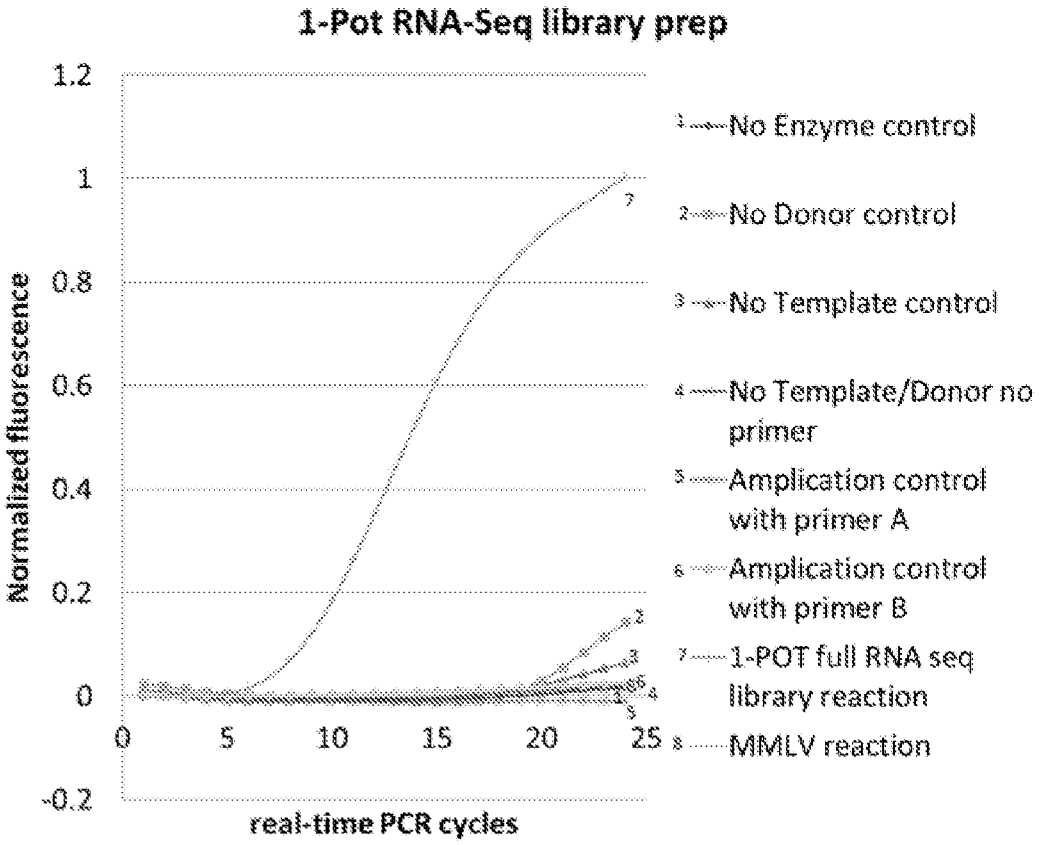


FIGURE 12B

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

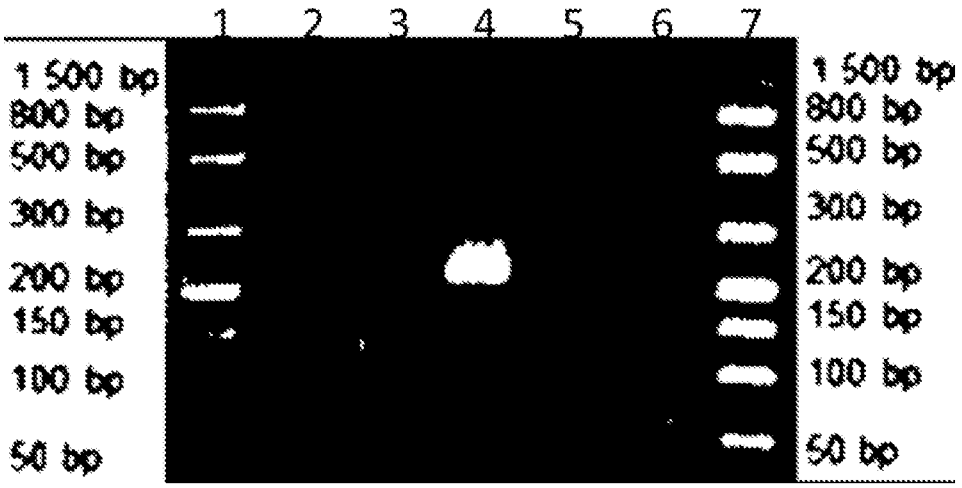


FIGURE 12C

Gel showing amplicon

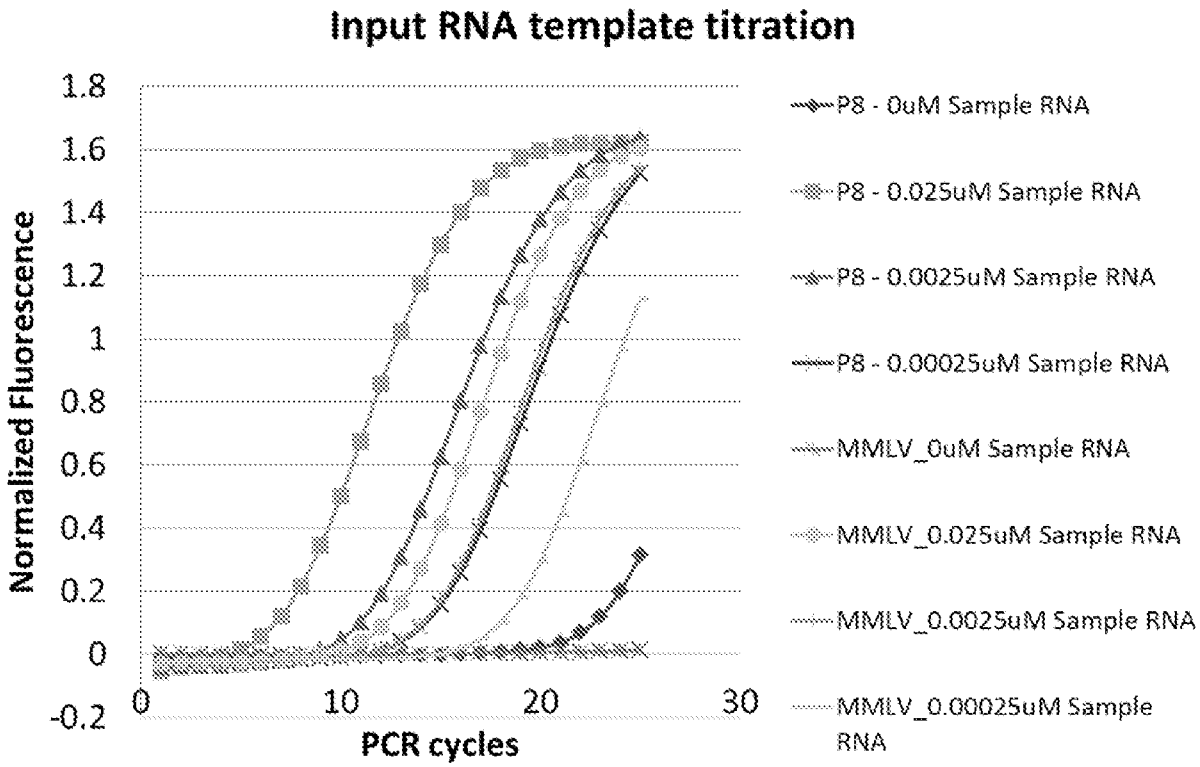


FIGURE 13

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

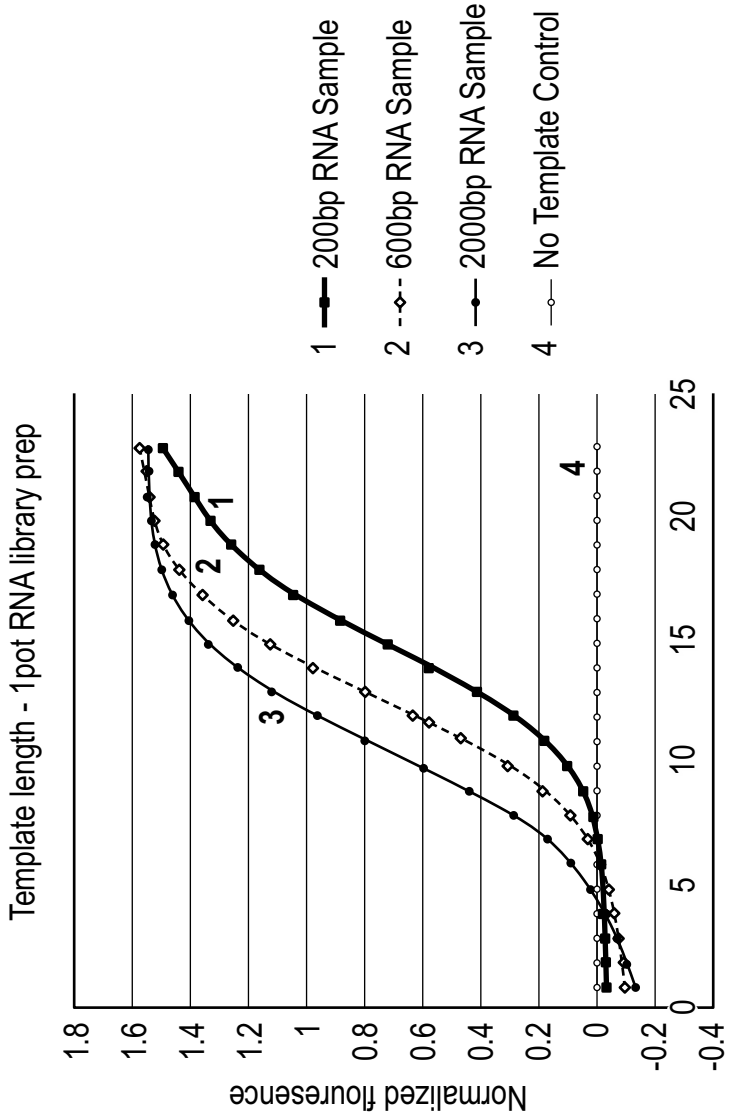


FIG. 14

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

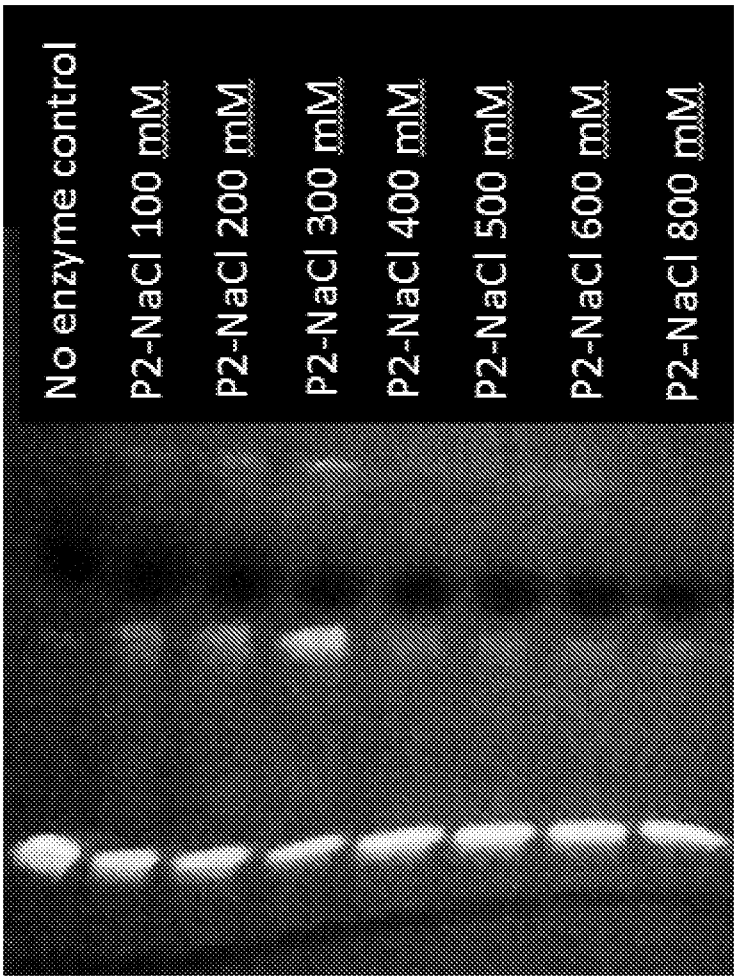


FIGURE 15

Enzyme activity and template jumping is dependent on NaCl concentration

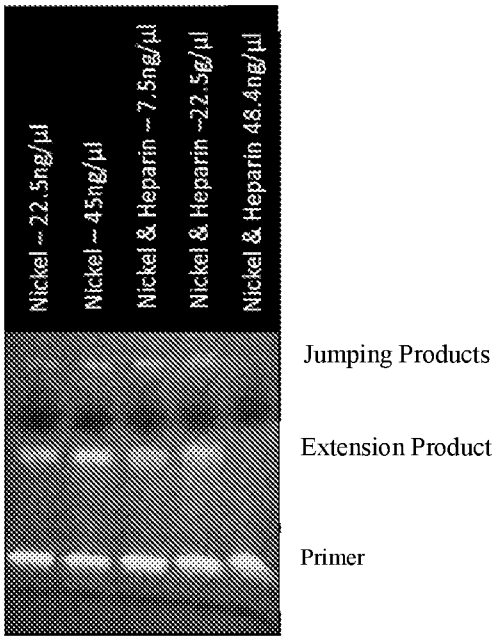


FIGURE 16A

Enzyme activity based on nickel and/or heparin affinity purification

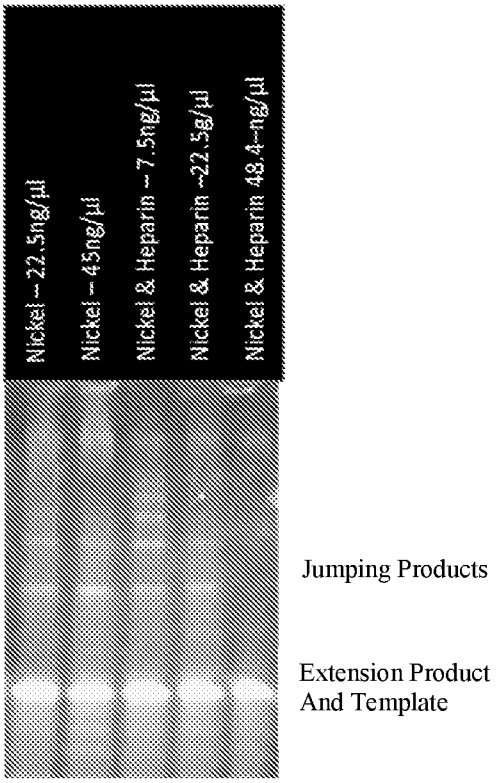


FIGURE 16B

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

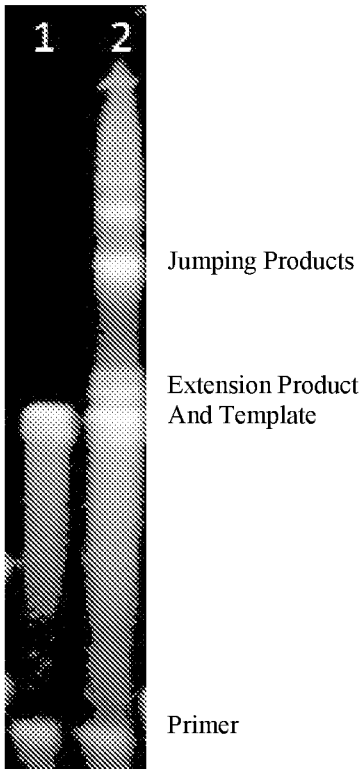


FIGURE 17

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)

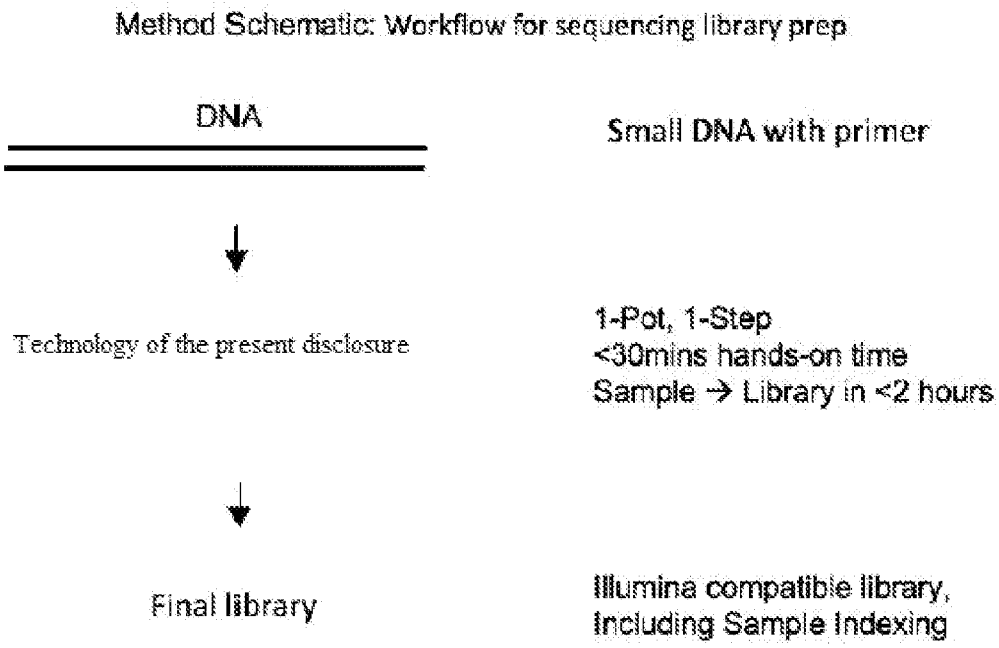


FIGURE 18A

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

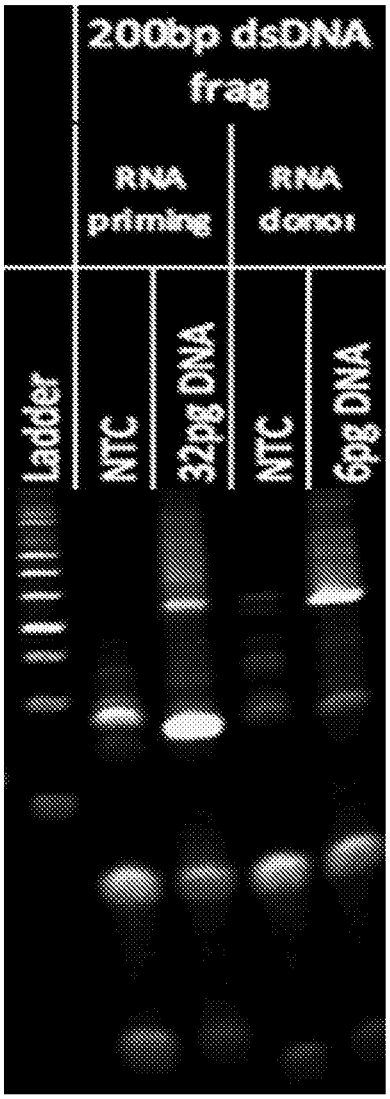


FIGURE 18B

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

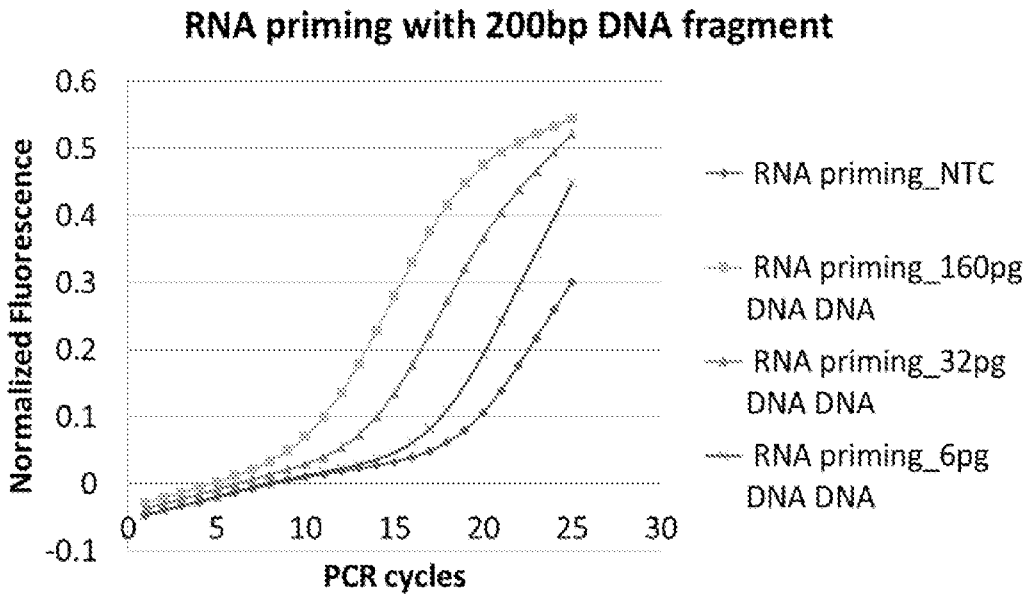


FIGURE 18C

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

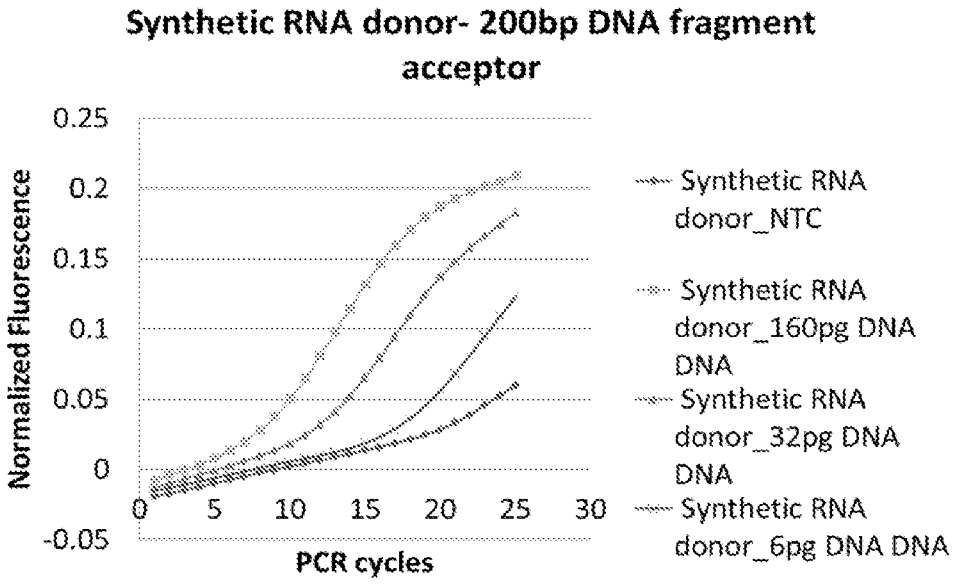


FIGURE 18D

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

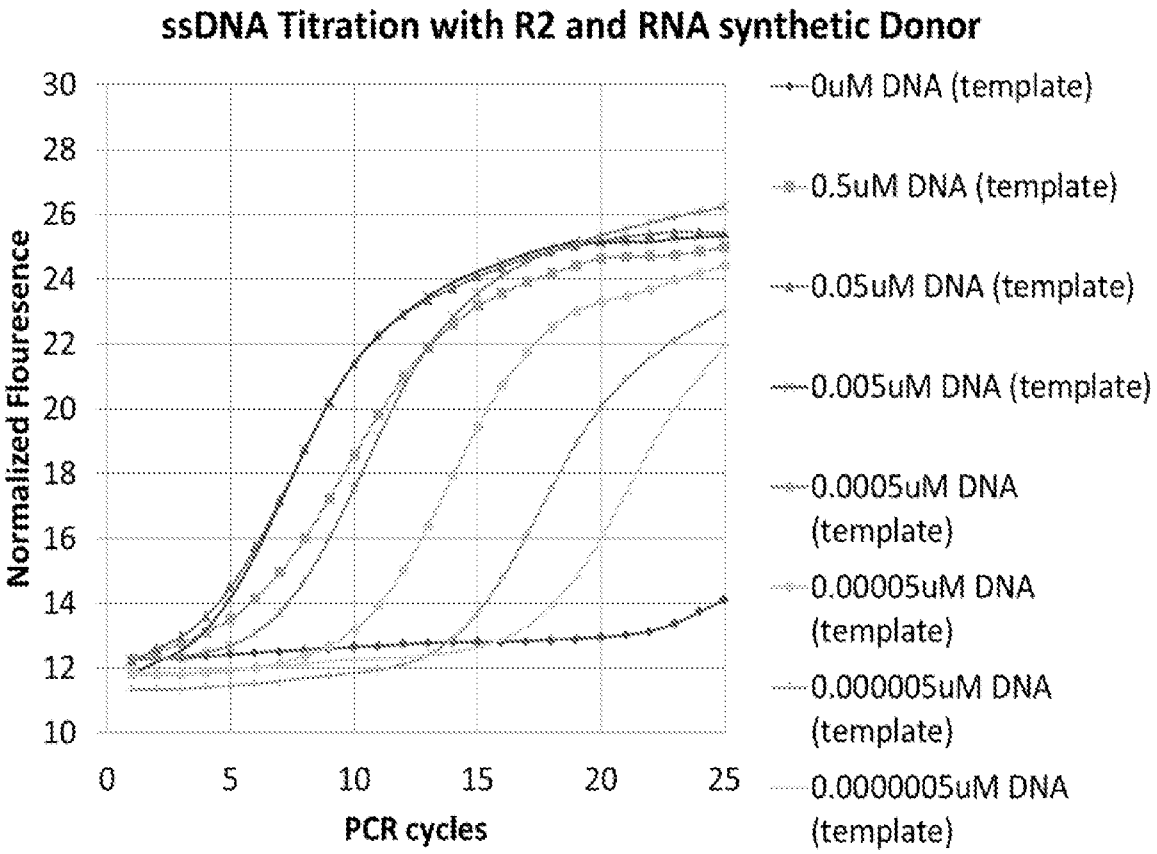


FIGURE 19

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

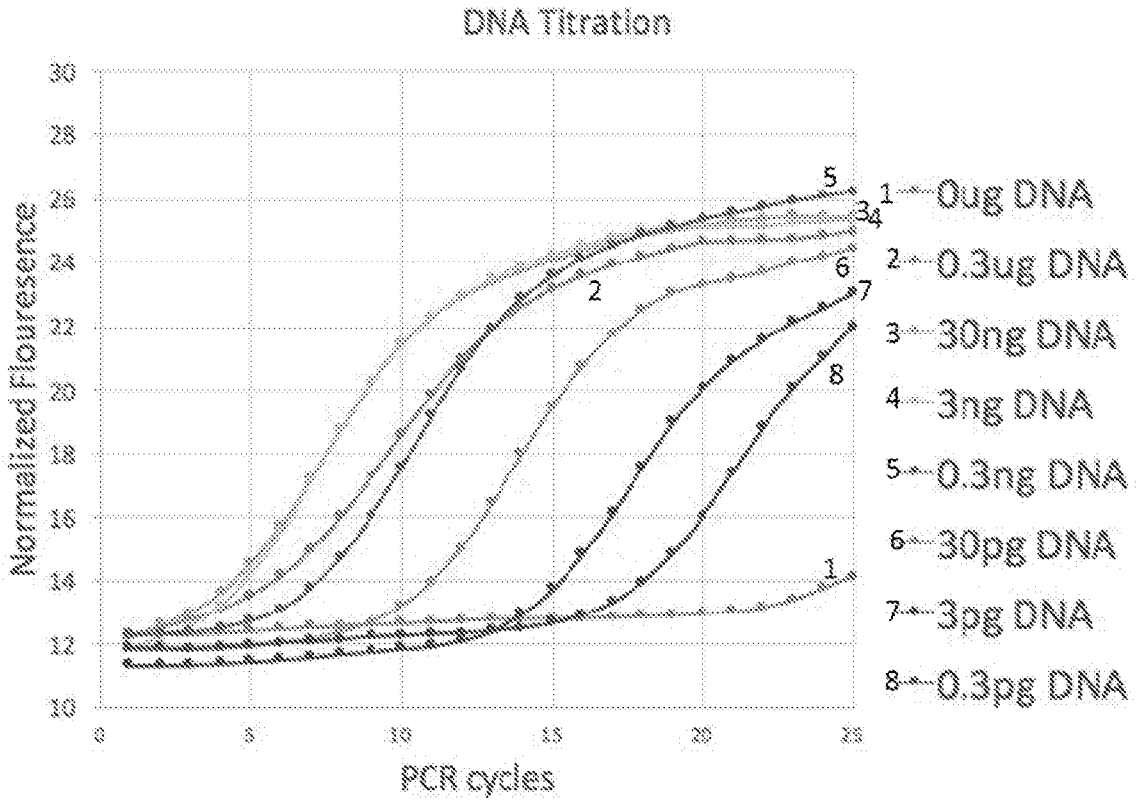


FIGURE 20

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

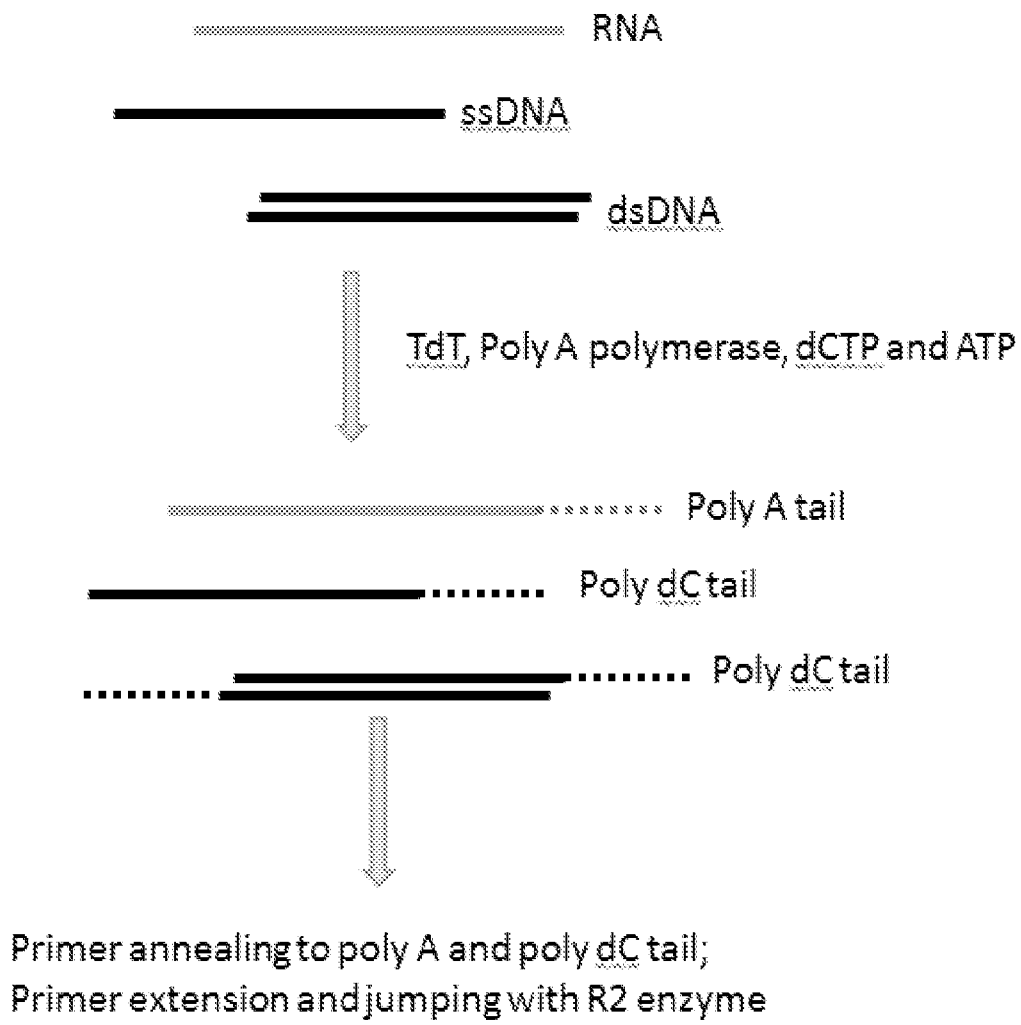


FIGURE 21

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

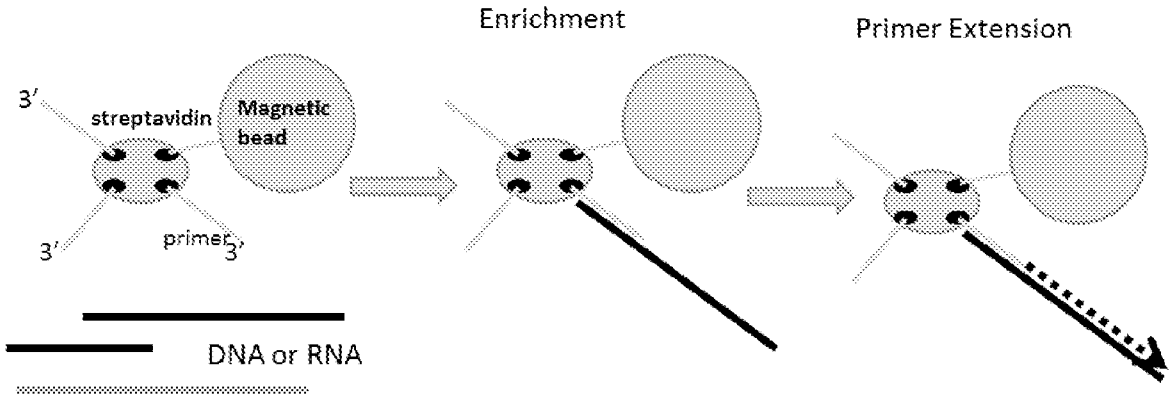


FIGURE 22A

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).

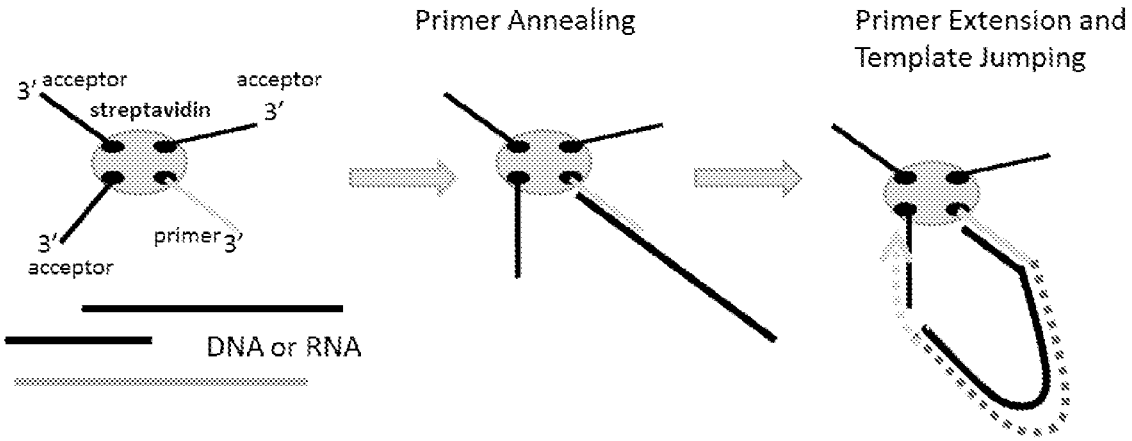


FIGURE 22B

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.

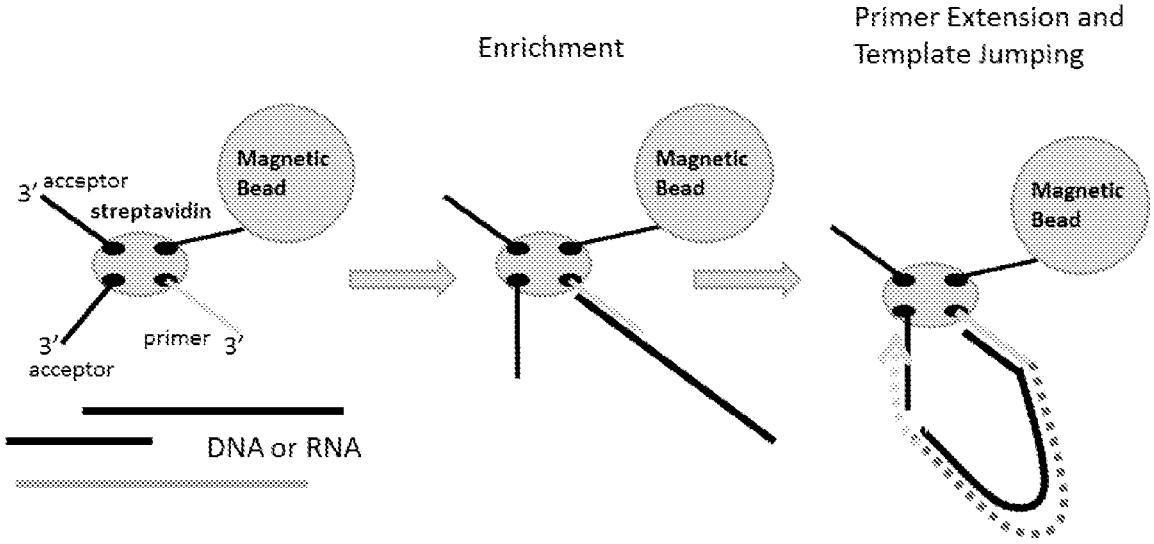


FIGURE 22C

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

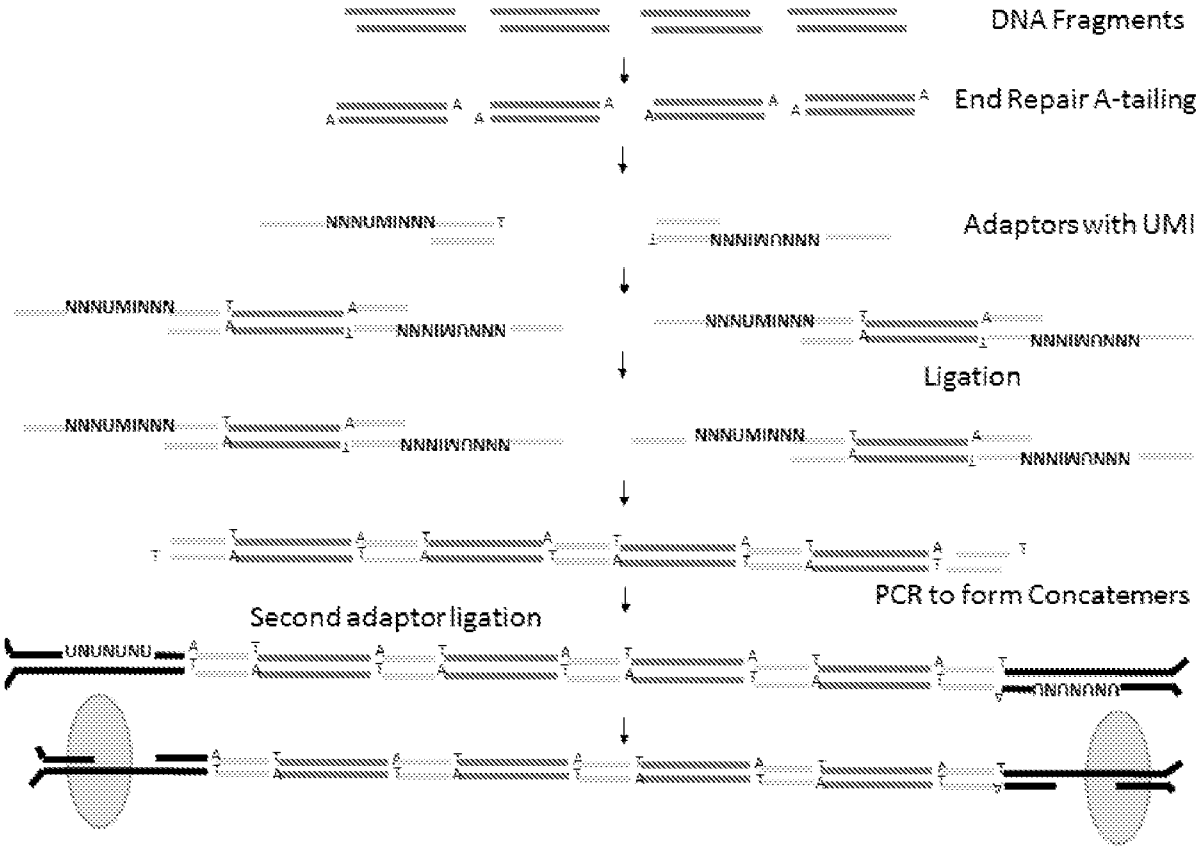


FIGURE 23A

Template Concatemerization



FIG. 23B

Product of a concatemerization that includes specific adaptors on both ends

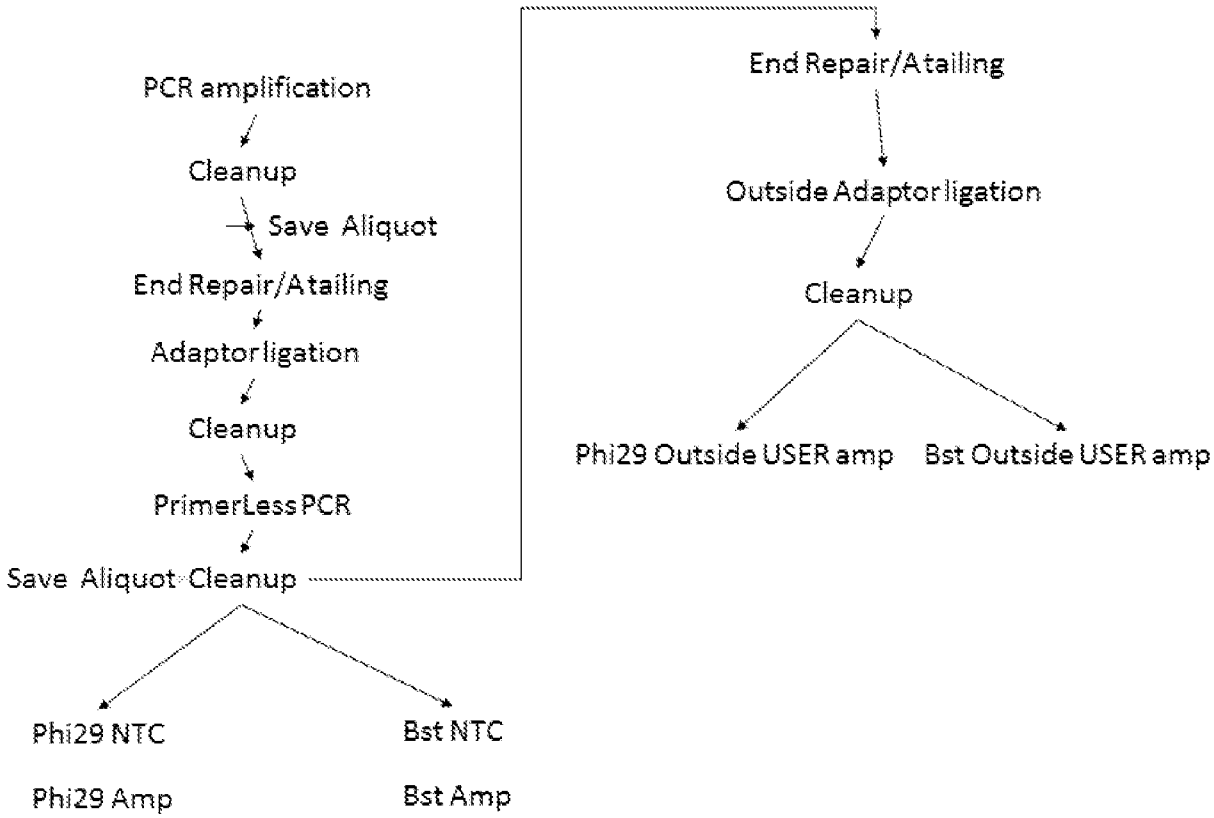


FIGURE 24A

Workflow of template concatemerization

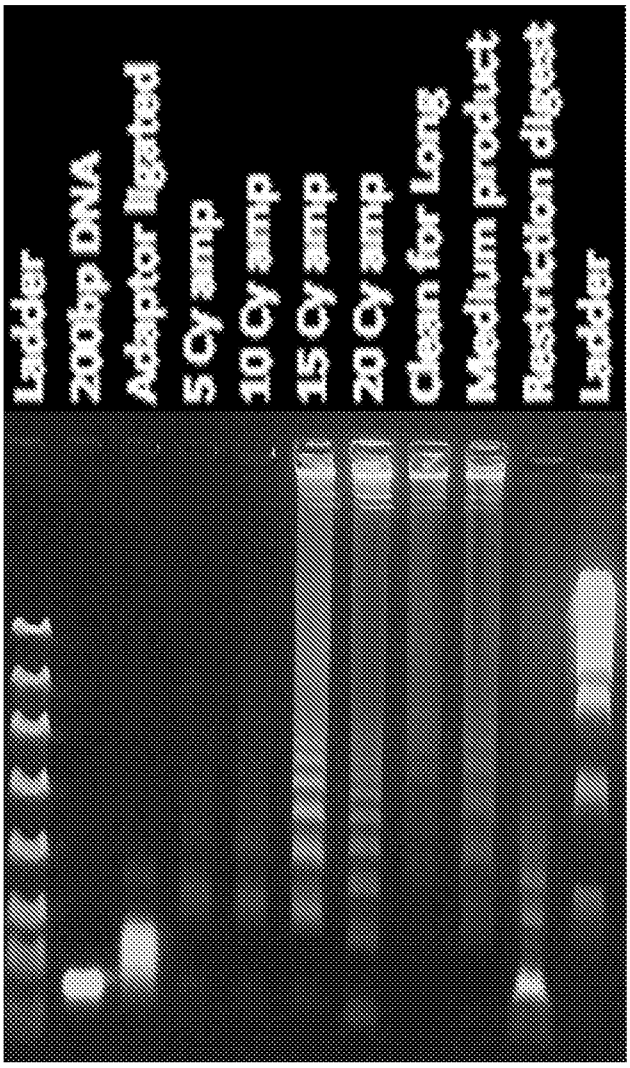


FIGURE 24B

Gel showing the concatemerization of a 200bp DNA fragment

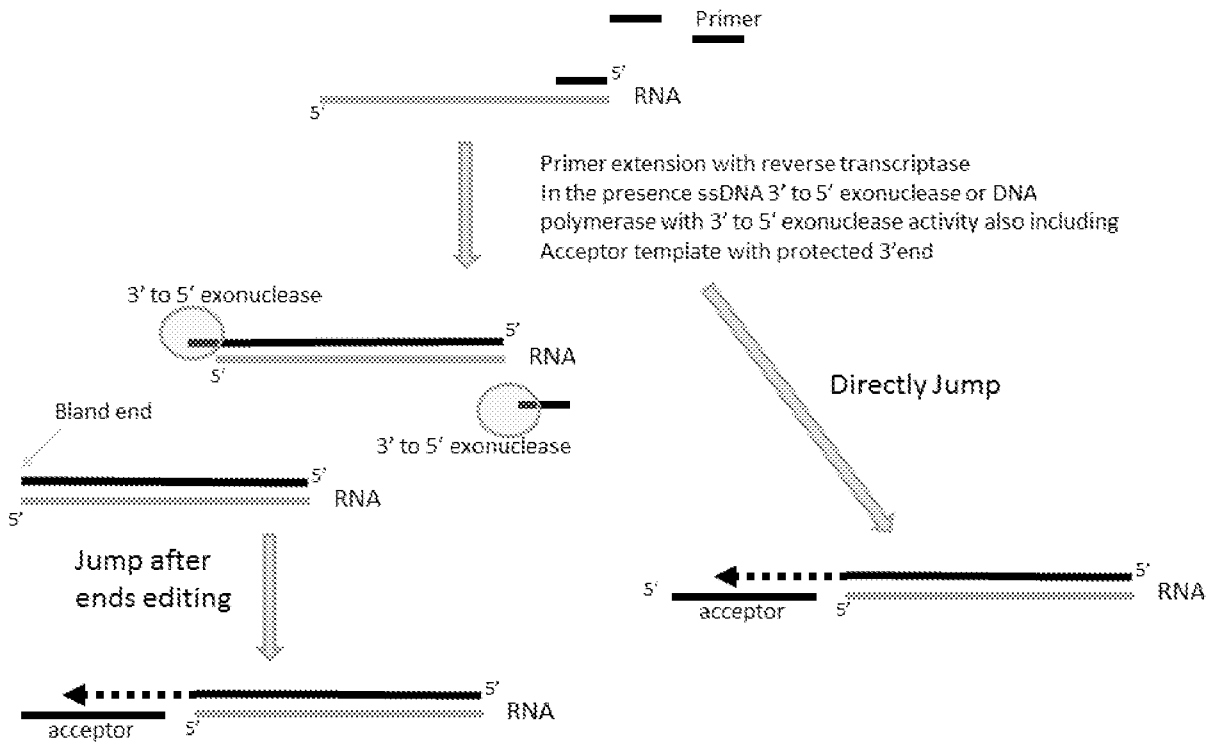


FIGURE 25

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

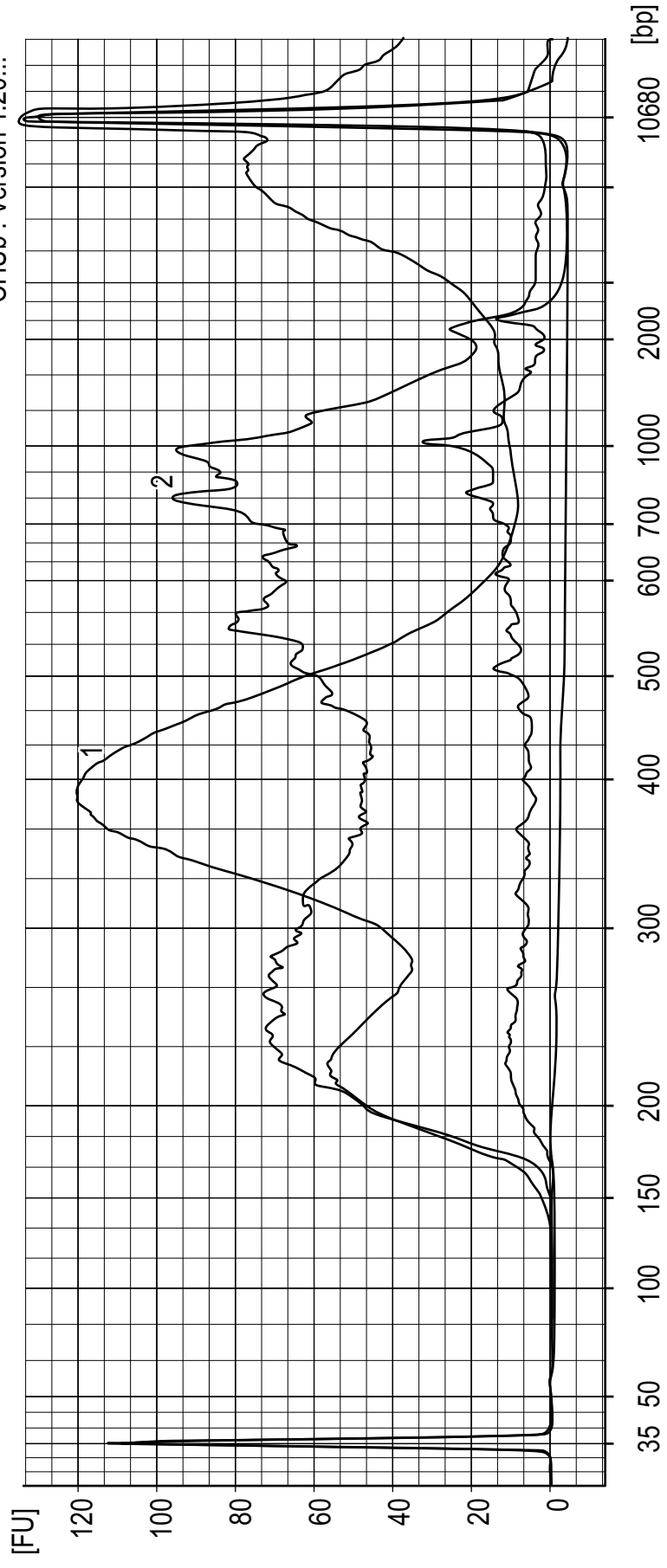


FIG. 26

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

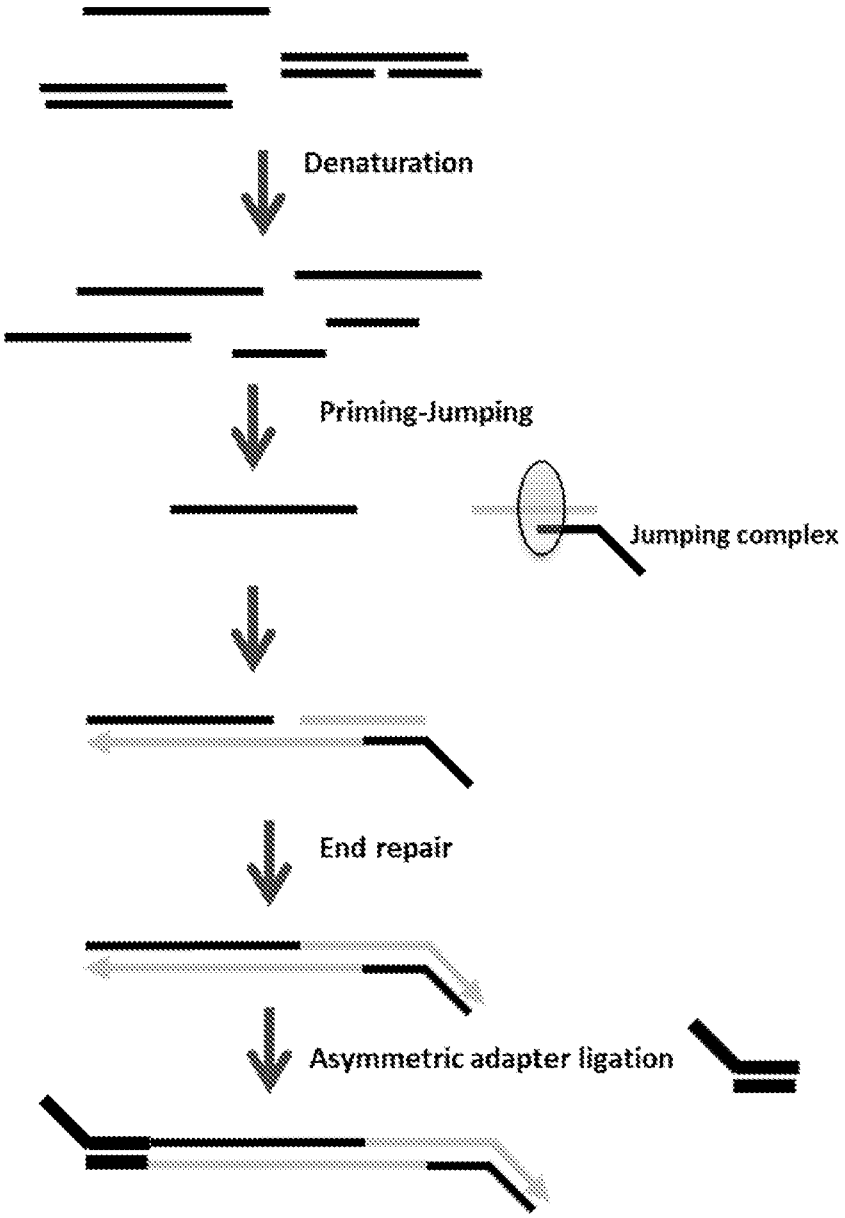


FIGURE 27

Method of generating cfDNA library

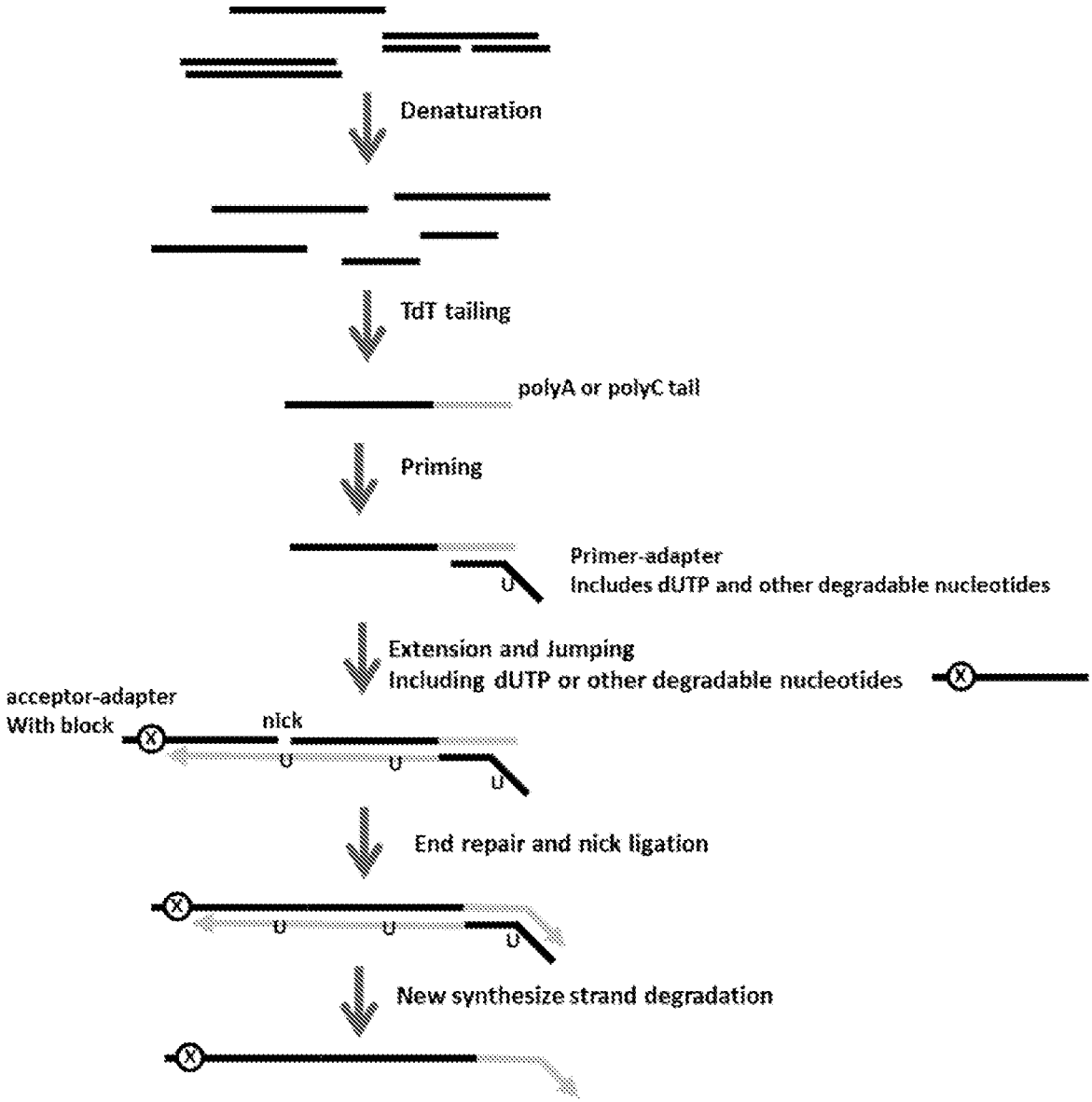


FIGURE 28

Method of generating cfDNA library

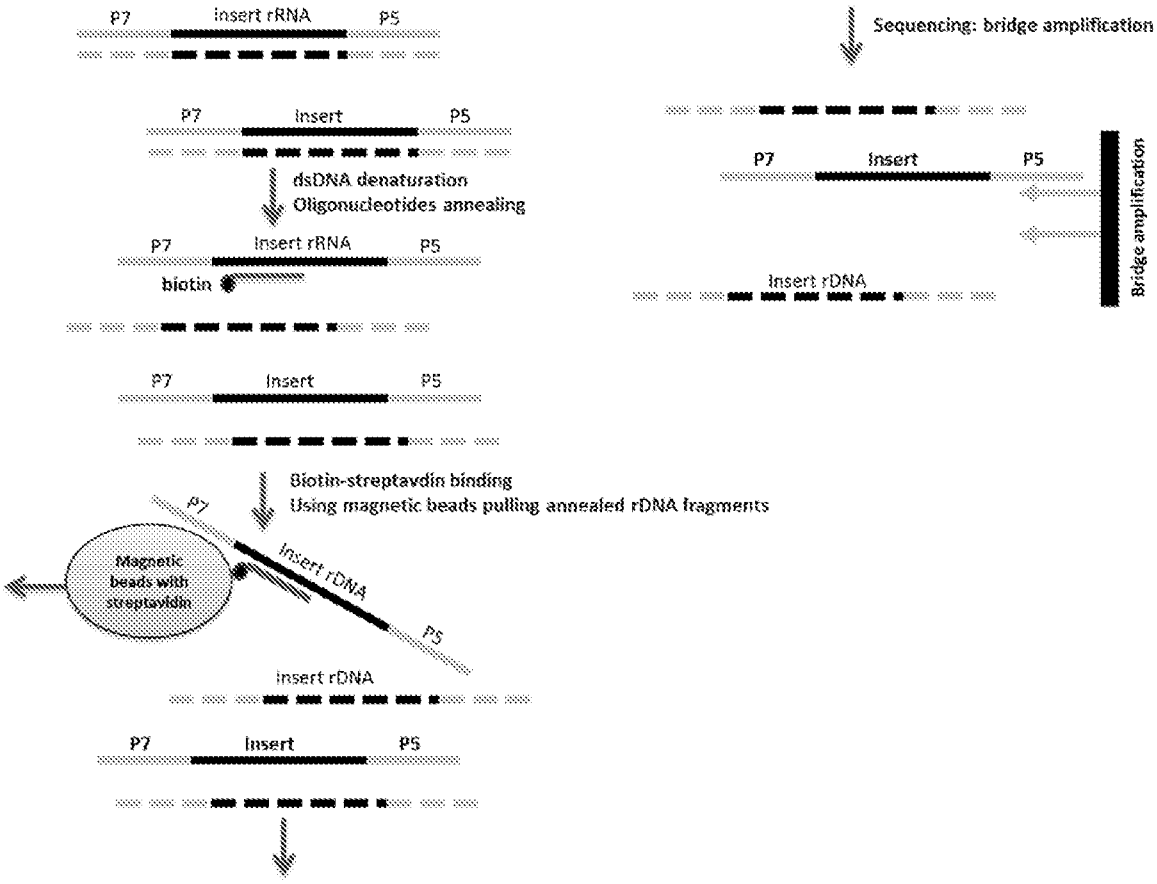


FIGURE 29

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

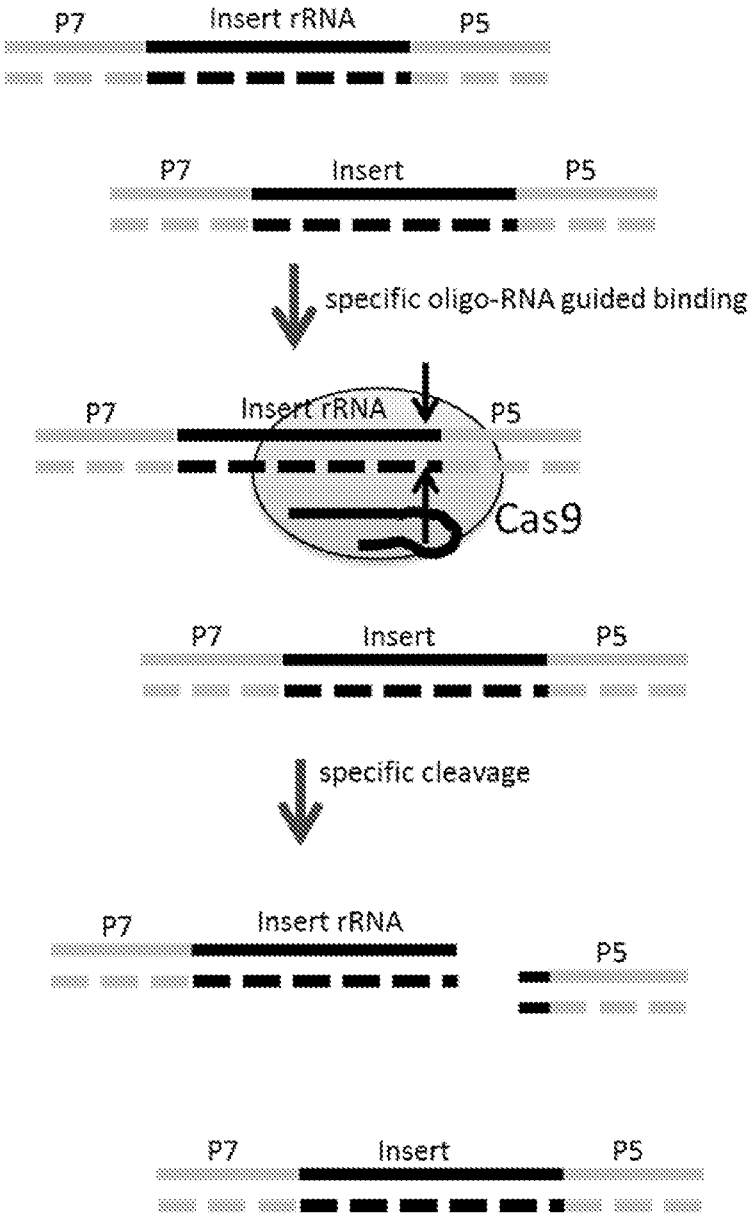
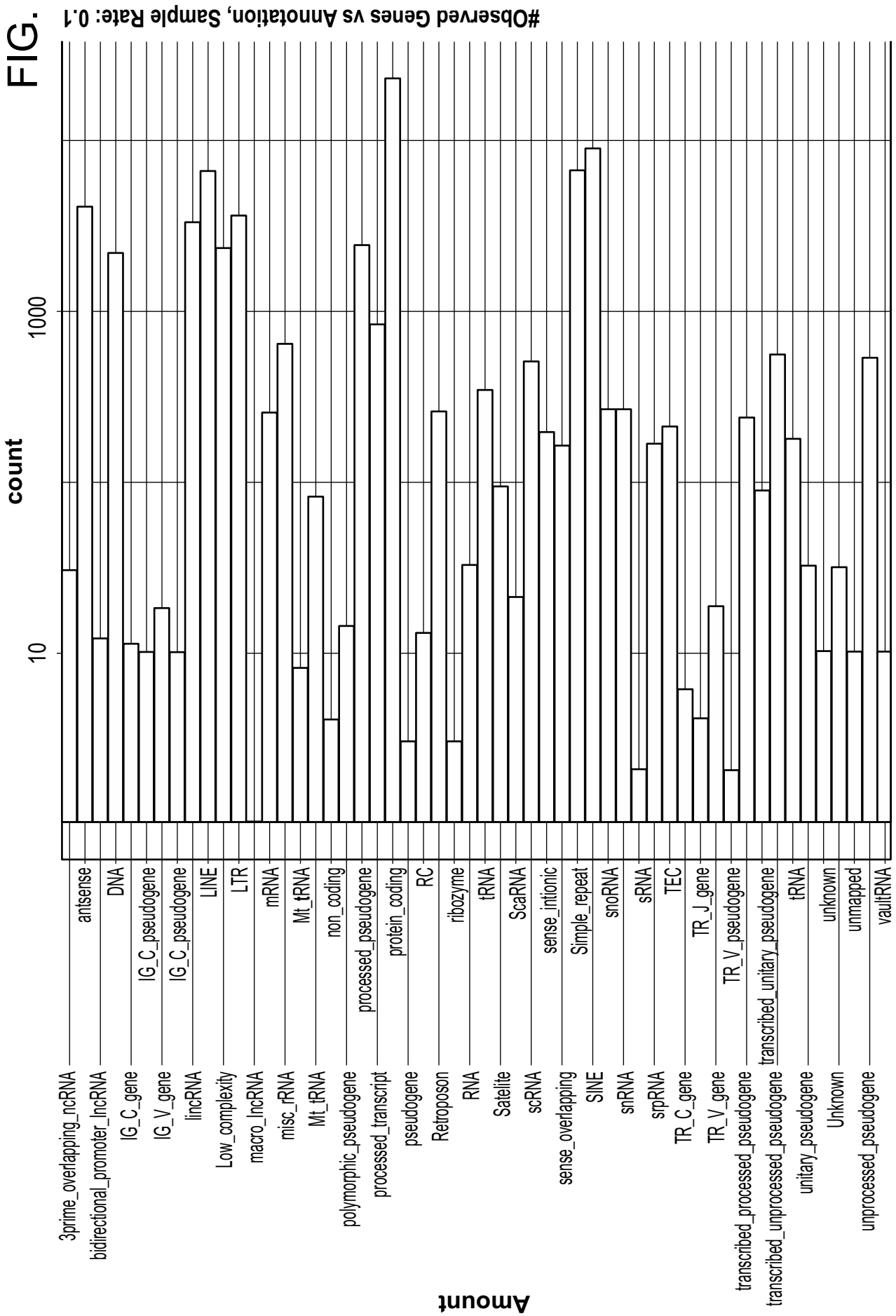


FIGURE 30

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

FIG. 31


#Observed Genes vs Annotation, Sample Rate: 0.1
count
10
1000
Amount
3prime_overlapping_ncRNA
antsense
bidirectional_promoter_lncRNA
DNA
IG_C_gene
IG_C_pseudogene
IG_V_gene
IG_C_pseudogene
lincRNA
LINE
Low_complexity
LTR
macro_lncRNA
mRNA
misc_rRNA
Mt_tRNA
Mt_tRNA
non_coding
polymorphic_pseudogene
processed_pseudogene
processed_transcript
protein_coding
pseudogene
RC
Retroposon
ribozyme
RNA
tRNA
Satelite
ScaRNA
scRNA
sense_intionic
sense_overlapping
Simple_repeat
SINE
snoRNA
snRNA
sRNA
srpRNA
TEC
TR_C_gene
TR_J_gene
TR_V_gene
TR_V_pseudogene
transcribed_processed_pseudogene
transcribed_unitary_pseudogene
transcribed_unprocessed_pseudogene
tRNA
unitary_pseudogene
unknown
Unknown
unmapped
unprocessed_pseudogene
vaultRNA