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(54) **INTEGRATIVE DNA AND RNA LIBRARY PREPARATIONS AND USES THEREOF**

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(57) **ABSTRACT**

The invention relates to integrated DNA and RNA library preparations and methods of making and uses thereof. The methods do not require physical separation of DNA and RNA. The methods output two separate libraries from DNA and RNA, respectively, which helps flexible manipulation on downstream sequencing platform.

Specification includes a Sequence Listing.

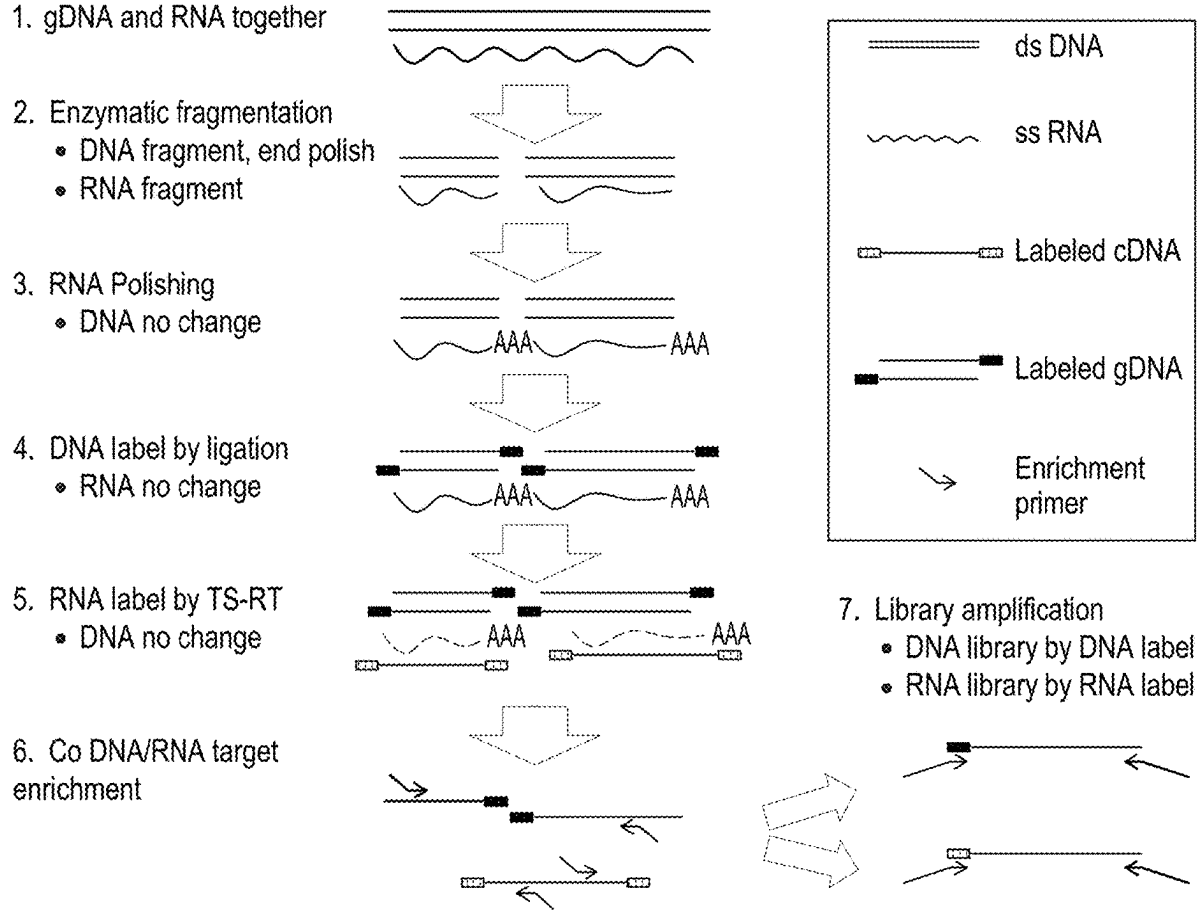
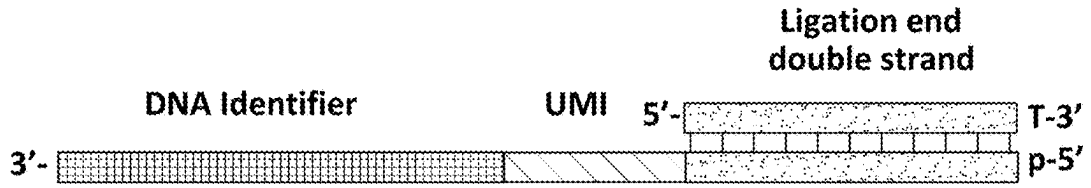
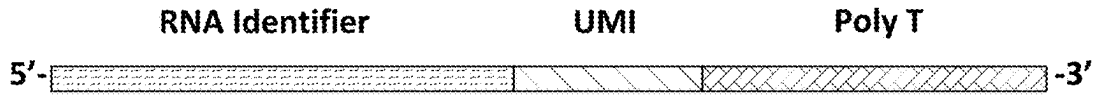


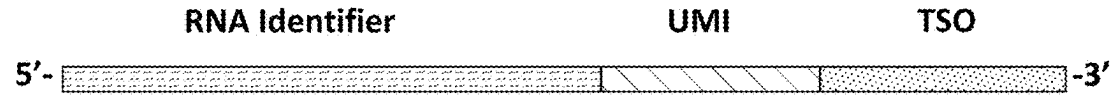
FIG. 1



DNA tag molecule that can be ligated to 3' end of double strand DNA fragments.

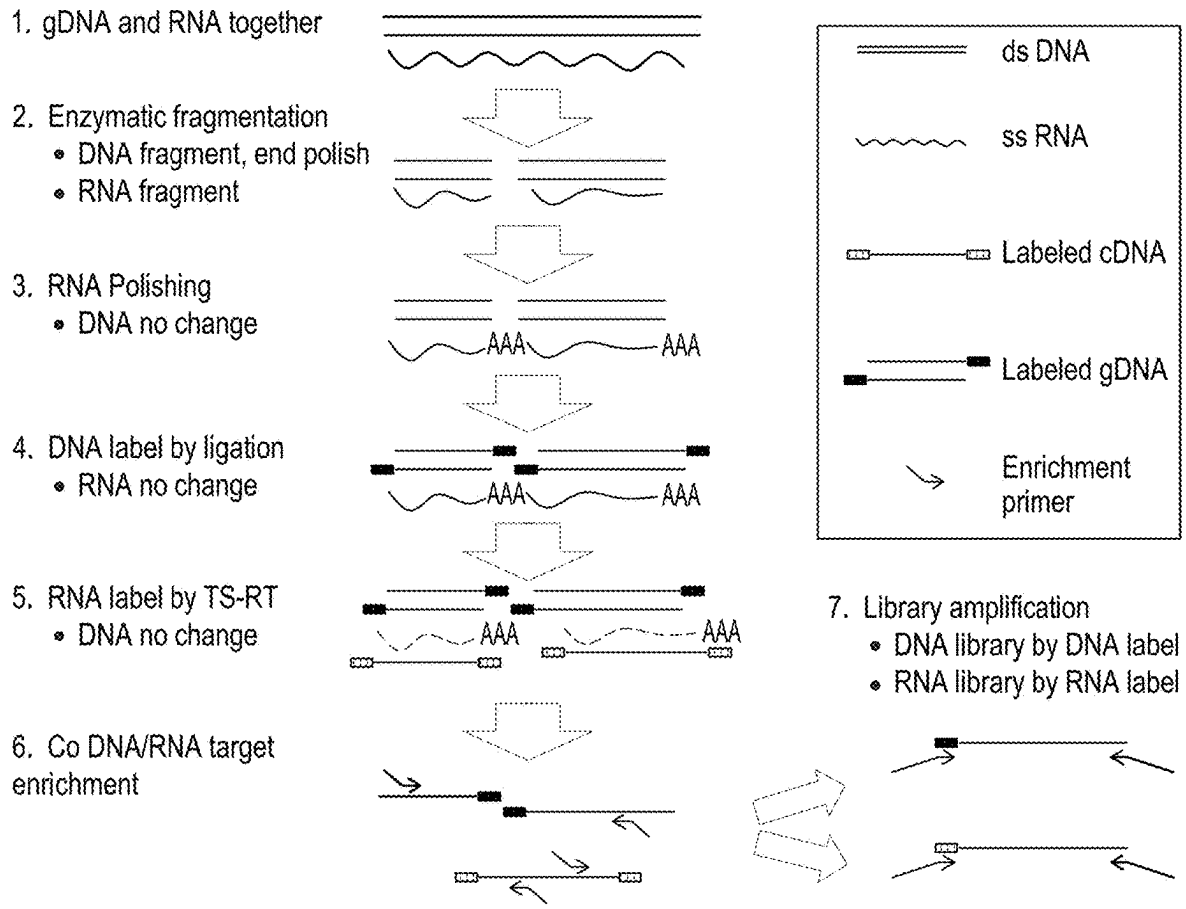


RNA tag molecule that can be used to add a 5' tag to RNA-derived cDNA fragments through reverse transcription.



RNA tag molecule that can be used to add a 3' tag to RNA-derived cDNA through template switching in RT.

FIG. 2


1. gDNA and RNA together
2. Enzymatic fragmentation
• DNA fragment, end polish
• RNA fragment
3. RNA Polishing
• DNA no change
AAA
AAA
4. DNA label by ligation
• RNA no change
AAA
AAA
5. RNA label by TS-RT
• DNA no change
AAA
AAA
6. Co DNA/RNA target enrichment
ds DNA
ss RNA
Labeled cDNA
Labeled gDNA
Enrichment primer
7. Library amplification
• DNA library by DNA label
• RNA library by RNA label

INTEGRATIVE DNA AND RNA LIBRARY PREPARATIONS AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application is a divisional of U.S. application Ser. No. 17/041,724 (now allowed), which is a 35 U.S.C. § 371 national phase of International Appl. No. PCT/US2019/024107 having an international filing date of Mar. 26, 2019, which claims benefit of U.S. Appl. No. 62/648,174, filed Mar. 26, 2018, the disclosure of each incorporated herein by reference in its entirety.

REFERENCE TO SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0002] The content of the electronically submitted sequence listing in XML format (Name: 24950002US02SEQL.xml; Size: 585,728 bytes; and Date of Creation: Dec. 30, 2024) filed with this application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

[0003] Paired DNA and RNA profiling enables researchers to gain more biological insights regarding the correlation between genotype and phenotype using samples from the same set of cell population.

[0004] To fully understand complex biological systems, researchers are getting more and more interested in multi-omic information, a full view of genomic, transcriptomic and proteomic data and their interactions. As the first step, paired DNA and RNA profiling are made possible with the advancement of sequencing technology. Traditional approaches, however, usually required preparing DNA and RNA samples in parallel, which meant the data obtained were not necessarily from the same set of cell population. Thus, these separate workflows might yield less correlative DNA and RNA data due to the heterogeneity of the sample. In addition, they added extra time and effort because of doubled workload.

[0005] In order to circumvent the disadvantage of separate workflow, researchers tried to integrate them. DR-seq (Dey S S et ah, Nat. Biotechnol. 33: 285-289 (2015)) and G&T-seq (Macaulay I C et ah, Nat. Methods. 72:519-22 (2015)) were among the first few attempts of integrative analysis of genomic DNA and mRNA from a single cell. However, these methods were designed specifically for single cell applications.

[0006] Another group developed an integrated DNA and RNA sequencing workflow, named Simul-seq. Reuter J A et al, Nat. Methods 75:953-958 (2016). It is a streamlined approach to profiling whole genome and transcriptome from the same set of cell population. Simul-seq is designed for whole genome and transcriptome sequencing.

[0007] There remains a need for improved, integrated DNA and RNA preparations amenable for sequencing analysis.

BRIEF SUMMARY OF THE INVENTION

[0008] Disclosed herein are methods for preparing DNA and cDNA libraries from a sample, comprising: ligating a DNA tag to an end of a DNA molecule in a sample, wherein the DNA tag comprises a unique molecular identifier (UMI) and a DNA identifier; and performing reverse transcription of a RNA molecule in the sample in the presence of a RNA tag, wherein the RNA tag comprises a RNA identifier, a UMI, and a poly(T).

[0009] In some embodiments, the reverse transcription is performed in the presence of a second RNA tag, wherein the second RNA tag comprises a RNA identifier, a UMI, and a template switching oligonucleotide (TSO).

[0010] In some embodiments, the methods further comprise amplifying the tagged DNA and tagged cDNA for enrichment with a set of gene specific primers. In some embodiments, the methods further comprise separating the amplified sample into first and second samples.

[0011] In some embodiments, the DNA and RNA molecules are obtained from a biological sample. In some embodiments, the DNA and RNA molecules are fragmented DNA and RNA from the biological sample.

[0012] In some embodiments, the DNA molecule contains polished ends for ligation. In some embodiments, the RNA molecule is polyadenylated.

[0013] In some embodiments, the methods do not require ribosomal depletion.

[0014] In further embodiments, the methods further comprise further amplifying the first sample with primers specific for the DNA tag. The amplification can generate a DNA library corresponding to the DNA molecules in the sample.

[0015] In further embodiments, the methods further comprise further amplifying the second sample with primers specific for the RNA tag. The amplification generates a cDNA library corresponding to the RNA molecules in a sample.

[0016] In some embodiments, the methods further comprise sequencing the DNA or cDNA library. The DNA library can be used for, but not limited to, DNA variant detection, copy number analysis, fusion gene detection, or structural variant detection. The cDNA library can be used for, but not limited to, RNA variant detection, gene expression analysis, or fusion gene detection. The libraries can be also used for paired DNA and RNA profiling.

[0017] Also disclosed herein are DNA libraries made by the methods disclosed herein. Further disclosed are cDNA libraries made by the methods disclosed herein.

[0018] Also disclosed herein are DNA tags comprising a unique molecular identifier (UMI) and a DNA identifier. In some embodiments, in the DNA tags, the UMI and the DNA identifier can be positioned in a 5' to 3' direction.

[0019] Also disclosed herein are RNA tags comprising a RNA identifier, a UMI, and a poly(T). In some embodiments, in the RNA tags, the RNA identifier, the UMI, and the poly(T) are positioned in a 5' to 3' direction. Also disclosed herein are RNA tags comprising a RNA identifier, a UMI, and a template switching oligonucleotide (TSO). In some embodiments, in the RNA tags, the RNA identifier, the UMI, and the TSO are positioned in a 5' to 3' direction.

[0020] Disclosed herein are compositions comprising at least 2 of the above described tags. Also disclosed herein are compositions comprising the DNA tag and the 2 different RNA tags as described above.

[0021] Further disclosed herein are methods for preparing targeted DNA and cDNA libraries, comprising:

[0022] (a) obtaining purified DNA and RNA from a biological sample;

[0023] (b) fragmenting the DNA and RNA;

[0024] (c) polishing the ends of the double stranded DNA fragments for ligation;

[0025] (d) polishing the RNA fragments by polyadenylation;

[0026] (e) ligating a DNA tag to a 3' end of the polished DNA fragments, wherein the DNA tag comprises in a 5' to 3' direction a unique molecular identifier (UMI) and a DNA identifier;

[0027] (f) performing reverse transcription of the polished RNA fragments in the presence of a first RNA tag, wherein the first RNA tag comprises in a 5' to 3' direction a RNA identifier, a UMI, and a poly(T), and a second RNA tag, wherein the second RNA tag comprises in a 5' to 3' direction a RNA identifier, a UMI, and a template switching oligonucleotide (TSO);

[0028] (g) amplifying the tagged DNA and tagged cDNA for enrichment with a set of gene specific primers;

[0029] (h) separating the amplified sample into first and second samples;

[0030] (i) amplifying the first sample with primers specific for the DNA tag; and

[0031] (j) amplifying the second sample with primers specific for the RNA tag.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

[0032] FIG. **1**. Exemplary DNA and RNA tag molecules.

[0033] FIG. **2**. Exemplary process for generating DNA and cDNA libraries.

DETAILED DESCRIPTION OF THE INVENTION

[0034] Disclosed herein are innovative approaches for integrative DNA and cDNA library preparations for analysis, such as by next-generation sequencing (NGS) analysis, without physical separation of DNA and RNA. These approaches integrate UMI (unique molecular index) technology and optionally, targeted enrichment technology, seamlessly into the workflow, which improve utilization of sequencing capacity and accuracy of the results. In addition, these methods output two separate DNA and cDNA libraries from DNA and RNA, respectively, which allow flexible manipulation on downstream sequencing platform. Compared to standalone DNA library and cDNA library methods, these approaches reduce sample consumption, simplify the experimental process, and can help researchers gain biological insights in genotype and phenotype correlations and molecular mechanisms of diseases.

[0035] Methods are described herein to prepare targeted DNA and cDNA libraries without the necessity of physical separation of genomic DNA (gDNA) and mRNA. The process involves three modules: (1) assign different DNA and RNA tag molecules to each individual DNA and RNA fragment, respectively, without separating them in the system; optionally, (2) amplify and enrich a subset of the tagged DNA and RNA fragments (target enrichment); and (3) differentially PCR amplify the tagged DNA and tagged cDNA in the (enriched) product to output two libraries corresponding to the original DNA and RNA, respectively.

[0036] The DNA and RNA tag molecules used in the first module are oligonucleotides comprising at least 1) an identifying sequence to distinguish a DNA library or RNA library, and 2) a UMI sequence for identifying each individual nucleic acid molecule.

[0037] The DNA and RNA tags are essential for the final separation of DNA and cDNA libraries in module 3, where they can serve as specific amplification primer sites for DNA and RNA. The UMI sequence helps improve accuracy for both DNA and RNA NGS analysis. Exemplary tag molecules are illustrated in FIG. **1**.

[0038] Two types of RNA tag molecules can be used in order to sequence the single stranded RNA from both directions, and thus, two different mechanisms can be used to attach the RNA specific sequence. Only one type of DNA tag molecule is needed because the DNA tag molecule can be ligated to both ends of the double stranded DNA.

[0039] The targeted enrichment reaction (module 2) enables focused view on relevant regions of interest and provides economic utilization of NGS sequencing capacity. It also mitigates the necessity for extra treatment of the sample associated with whole genome or transcriptome workflow, such as ribosomal RNA depletion. The enrichment is done in the same reaction for both DNA and RNA. Depending on the applications, the enrichment primer pool can be the same if the target DNA and RNA regions are the same. If different regions are of interest for the DNA and RNA, users can simply mix the corresponding enrichment primer pools, and put them into the same reaction.

[0040] Module 3 enables separated output of DNA and cDNA libraries. The sequencing depth requirements for DNA and cDNA are usually quite different, and they vary depending on the applications. The output from the methods disclosed herein gives users flexibility so that sequencing capacity can be allocated individually according to specific needs. In addition, since the samples have already been partially amplified in module 2, the separation has negligible effect on sample loss.

[0041] FIG. **2** illustrates one exemplary, optimized way to utilize the methods disclosed herein. It starts with purified (not necessarily separated) gDNA and RNA from a biological sample (step 1). The total nucleic acids are fragmented by enzymatic digestion (for DNA) and by heat hydrolysis (for RNA). The double stranded DNA fragments are end polished so that they are ready for ligation (step 2). The fragmented RNAs are end polished by polyadenylation (step 3). In the next few steps, DNA fragments are ligated to DNA tag molecules (step 4), and the RNA fragments are attached with RNA tag molecules (on both ends) by template switching reverse transcription (step 5). With both DNA and RNA tags in place, the sample is subjected to targeted enrichment reaction by a set of gene specific primers, in which the regions of interest are amplified and enriched (step 6). Finally, the sample is split into two samples, and further amplified by primers specific for the DNA tag and RNA tag, respectively, and with proper NGS adapter sequences compatible with, e.g., Illumina NGS platform (step 7). The final products are two separate DNA and cDNA libraries resulted from the original DNA and RNA material, respectively, and are ready for sequencing.

[0042] Disclosed herein are methods for preparing DNA and cDNA libraries from a sample, comprising: ligating a DNA tag to an end of a DNA molecule in a sample, wherein the DNA tag comprises a unique molecular identifier (UMI) and a DNA identifier; and performing reverse transcription of a RNA molecule in the sample in the presence of a RNA tag, wherein the RNA tag comprises a RNA identifier, a UMI, and a poly(T). The methods do not require physical separation of the DNA and RNA from the sample.

[0043] In some embodiments, the reverse transcription is performed in the presence of a second RNA tag, wherein the second RNA tag comprises a RNA identifier, a UMI, and a template switching oligonucleotide (TSO).

[0044] In some embodiments, the methods can include ribosomal depletion. Alternatively, in some embodiments, the methods do not require ribosomal depletion. Methods for ribosomal depletion are known in the art, e.g., using RiboZero gold (Illumina: MRZG126).

[0045] The term "sample" can include RNA, DNA, a single cell, multiple cells, fragments of cells, or an aliquot of body fluid, taken from a subject (e.g., a mammalian subject, an animal subject, a human subject, or a non-human animal subject). Samples can be selected by one of skill in the art using any known means known including but not limited to centrifugation, venipuncture, blood draw, excretion, swabbing, biopsy, needle aspirate, lavage sample, scraping, surgical incision, laser capture microdissection, gradient separation, or intervention or other means known in the art. The term "mammal" or "mammalian" as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[0046] As used herein, the term "biological sample" is intended to include, but is not limited to, tissues, cells, biological fluids and isolates thereof, isolated from a subject, as well as tissues, cells, and fluids present within a subject.

[0047] As used herein, a "single cell" refers to one cell. Single cells useful in the methods described herein can be obtained from a tissue of interest, or from a biopsy, blood sample, or cell culture. Additionally, cells from specific organs, tissues, tumors, neoplasms, or the like can be obtained and used in the methods described herein. In general, cells from any population can be used in the methods, such as a population of prokaryotic or eukaryotic organisms, including bacteria or yeast.

[0048] A single cell suspension can be obtained using standard methods known in the art including, for example, enzymatically using trypsin or papain to digest proteins connecting cells in tissue samples or releasing adherent cells in culture, or mechanically separating cells in a sample. Samples can also be selected by one of skill in the art using one or more markers known to be associated with a sample of interest.

[0049] Methods for manipulating single cells are known in the art and include fluorescence activated cell sorting (FACS), micromanipulation and the use of semi-automated cell pickers (e.g., the Quixell™ cell transfer system from Stoelting Co.). Individual cells can, for example, be individually selected based on features detectable by microscopic observation, such as location, morphology, or reporter gene expression.

[0050] Once a desired sample has been identified, the sample is prepared and the cell(s) are lysed to release cellular contents including DNA and RNA, such as gDNA and mRNA, using methods known to those of skill in the art. Lysis can be achieved by, for example, heating the cells, or by the use of detergents or other chemical methods, or by a combination of these. Any suitable lysis method known in the art can be used.

[0051] Nucleic acids from a cell such as DNA or RNA are isolated using methods known to those of skill in the art.

[0052] The term "polynucleotide(s)" or "oligonucleotide (s)" refers to nucleic acids such as DNA molecules and RNA molecules and analogs thereof (e.g., DNA or RNA generated using nucleotide analogs or using nucleic acid chemistry). As desired, the polynucleotides can be made synthetically, e.g., using art-recognized nucleic acid chemistry or enzymatically using, e.g., a polymerase, and, if desired, can be modified. Typical modifications include methylation, biotinylation, and other art-known modifications. In addition, a polynucleotide can be single-stranded or double-stranded and, where desired, linked to a detectable moiety. In some aspects, a polynucleotide can include hybrid molecules, e.g., comprising DNA and RNA.

[0053] "G," "C," "A," "T" and "U" each generally stands for a nucleotide that contains guanine, cytosine, adenine, thymidine and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base can base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine can be replaced in nucleotide sequences by a nucleotide containing, for example, inosine. In another example, adenine and cytosine anywhere in the oligonucleotide can be replaced with guanine and uracil, respectively, to form G-U Wobble base pairing with the target mRNA. Sequences containing such replacement moieties are suitable for the compositions and methods described herein.

[0054] The term "DNA" refers to chromosomal DNA, plasmid DNA, phage DNA, or viral DNA that is single stranded or double stranded. DNA can be obtained from prokaryotes or eukaryotes.

[0055] The term "genomic DNA" or gDNA" refers to chromosomal DNA.

[0056] The term "messenger RNA" or "mRNA" refers to an RNA that is without introns and that can be translated into a polypeptide.

[0057] The term "cDNA" refers to a DNA that is complementary or identical to an mRNA, in either single stranded or double stranded form.

[0058] As used herein, "polymerase" and its derivatives, generally refers to any enzyme that can catalyze the polymerization of nucleotides (including analogs thereof) into a nucleic acid strand. Typically. but not necessarily, such nucleotide polymerization can occur in a template-dependent fashion. Such polymerases can include without limitation naturally occurring polymerases and any subunits and truncations thereof, mutant polymerases, variant polymerases, recombinant, fusion or otherwise engineered polymerases, chemically modified polymerases, synthetic molecules or assemblies, and any analogs, derivatives or fragments thereof that retain the ability to catalyze such polymerization. Optionally, the polymerase can be a mutant polymerase comprising one or more mutations involving the replacement of one or more amino acids with other amino acids, the insertion or deletion of one or more amino acids from the polymerase, or the linkage of parts of two or more polymerases. Typically, the polymerase comprises one or more active sites at which nucleotide binding and/or catalysis of nucleotide polymerization can occur. Some exemplary polymerases include without limitation DNA polymerases

and RNA polymerases. The term "polymerase" and its variants, as used herein, also refers to fusion proteins comprising at least two portions linked to each other, where the first portion comprises a peptide that can catalyze the polymerization of nucleotides into a nucleic acid strand and is linked to a second portion that comprises a second polypeptide. In some embodiments, the second polypeptide can include a reporter enzyme or a processivity-enhancing domain. Optionally, the polymerase can possess 5' exonuclease activity or terminal transferase activity. In some embodiments, the polymerase can be optionally reactivated, for example through the use of heat, chemicals or re-addition of new amounts of polymerase into a reaction mixture. In some embodiments, the polymerase can include a hot-start polymerase or an aptamer based polymerase that optionally can be reactivated.

[0059] The term "extension" and its variants, as used herein, when used in reference to a given primer, comprises any in vivo or in vitro enzymatic activity characteristic of a given polymerase that relates to polymerization of one or more nucleotides onto an end of an existing nucleic acid molecule. Typically, but not necessarily such primer extension occurs in a template-dependent fashion; during template-dependent extension, the order and selection of bases is driven by established base pairing rules, which can include Watson-Crick type base pairing rules or alternatively (and especially in the case of extension reactions involving nucleotide analogs) by some other type of base pairing paradigm. In one non-limiting example, extension occurs via polymerization of nucleotides on the 3'OH end of the nucleic acid molecule by the polymerase.

[0060] As used herein, the terms "ligating," "ligation," and their derivatives refer generally to the act or process for covalently linking two or more molecules together, for example, covalently linking two or more nucleic acid molecules to each other. In some embodiments, ligation includes joining nicks between adjacent nucleotides of nucleic acids. In some embodiments, ligation includes forming a covalent bond between an end of a first and an end of a second nucleic acid molecule. In some embodiments, for example embodiments wherein the nucleic acid molecules to be ligated include conventional nucleotide residues, the ligation can include forming a covalent bond between a 5' phosphate group of one nucleic acid and a 3' hydroxyl group of a second nucleic acid thereby forming a ligated nucleic acid molecule. In some embodiments, any means for joining nicks or bonding a 5'phosphate to a 3' hydroxyl between adjacent nucleotides can be employed. In an exemplary embodiment, an enzyme such as a ligase can be used. Generally, for the purposes of this disclosure, an amplified target sequence can be ligated to an adapter to generate an adapter-ligated amplified target sequence.

[0061] As used herein, "ligase" and its derivatives, refers generally to any agent capable of catalyzing the ligation of two substrate molecules. In some embodiments, the ligase includes an enzyme capable of catalyzing the joining of nicks between adjacent nucleotides of a nucleic acid. In some embodiments, the ligase includes an enzyme capable of catalyzing the formation of a covalent bond between a 5' phosphate of one nucleic acid molecule to a 3' hydroxyl of another nucleic acid molecule thereby forming a ligated nucleic acid molecule. Suitable ligases can include, but not limited to, T4 DNA ligase, T4 RNA ligase, and *E. coli* DNA ligase.

[0062] As used herein, "ligation conditions" and its derivatives, generally refers to conditions suitable for ligating two molecules to each other. In some embodiments, the ligation conditions are suitable for sealing nicks or gaps between nucleic acids. As defined herein, a "nick" or "gap" refers to a nucleic acid molecule that lacks a directly bound 5' phosphate of a mononucleotide pentose ring to a 3' hydroxyl of a neighboring mononucleotide pentose ring within internal nucleotides of a nucleic acid sequence. As used herein, the term nick or gap is consistent with the use of the term in the art. Typically, a nick or gap can be ligated in the presence of an enzyme, such as ligase at an appropriate temperature and pH. In some embodiments, T4 DNA ligase can join a nick between nucleic acids at a temperature of about 70° C.-72° C.

[0063] As used herein, "blunt-end ligation" and its derivatives, refers generally to ligation of two blunt-end double-stranded nucleic acid molecules to each other. A "blunt end" refers to an end of a double-stranded nucleic acid molecule wherein substantially all of the nucleotides in the end of one strand of the nucleic acid molecule are base paired with opposing nucleotides in the other strand of the same nucleic acid molecule. A nucleic acid molecule is not blunt ended if it has an end that includes a single-stranded portion greater than two nucleotides in length, referred to herein as an "overhang." In some embodiments, the end of nucleic acid molecule does not include any single stranded portion, such that every nucleotide in one strand of the end is based paired with opposing nucleotides in the other strand of the same nucleic acid molecule. In some embodiments, the ends of the two blunt ended nucleic acid molecules that become ligated to each other do not include any overlapping, shared or complementary sequence. Typically, blunted-end ligation excludes the use of additional oligonucleotide adapters to assist in the ligation of the double-stranded amplified target sequence to the double-stranded adapter, such as patch oligonucleotides as described in Mitra and Varley, US2010/0129874. In some embodiments, blunt-ended ligation includes a nick translation reaction to seal a nick created during the ligation process.

[0064] The term "amplicon" refers to the amplified product of a nucleic acid amplification reaction, e.g., RT-PCR.

[0065] The terms "reverse-transcriptase PCR" and "RT-PCR" refer to a type of PCR where the starting material is mRNA. The starting mRNA is enzymatically converted to complementary DNA or "cDNA" using a reverse transcriptase enzyme. The cDNA is then used as a template for a PCR reaction.

[0066] The terms "PCR product," "PCR fragment," and "amplification product" refer to the resultant mixture of compounds after two or more cycles of the PCR steps of denaturation, annealing and extension are complete. These terms encompass the case where there has been amplification of one or more segments of one or more target sequences.

[0067] The term "amplification reagents" refers to those reagents (deoxyribonucleotide triphosphates, buffer, etc.), needed for amplification except for primers, nucleic acid template, and the amplification enzyme. Typically, amplification reagents along with other reaction components are placed and contained in a reaction vessel (test tube, microwell, etc.). Amplification methods include PCR methods known to those of skill in the art and also include rolling circle amplification (Blanco et al., J. Biol. Chem., 264,

8935-8940, 1989), hyperbranched rolling circle amplification (Lizard et al., Nat. Genetics, 19, 225-232, 1998), and loop-mediated isothermal amplification (Notomi et al., Nucl. Acids Res., 28, e63, 2000), each of which is hereby incorporated by reference in its entirety.

[0068] The term "hybridize" refers to a sequence specific non-covalent binding interaction with a complementary nucleic acid. Hybridization can occur to all or a portion of a nucleic acid sequence. Those skilled in the art will recognize that the stability of a nucleic acid duplex, or hybrids, can be determined by the Tm. Additional guidance regarding hybridization conditions can be found in: Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6 and in: Sambrook et al., Molecular Cloning, a Laboratory Manual, Cold Spring Harbor Laboratory Press, Vol. 3, 1989.

[0069] As used herein, "incorporating" a sequence into a polynucleotide refers to covalently linking a series of nucleotides with the rest of the polynucleotide, for example at the 3' or 5' end of the polynucleotide, by phosphodiester bonds, wherein the nucleotides are linked in the order prescribed by the sequence. A sequence has been "incorporated" into a polynucleotide, or equivalently the polynucleotide "incorporates" the sequence, if the polynucleotide contains the sequence or a complement thereof. Incorporation of a sequence into a polynucleotide can occur enzymatically (e.g., by ligation or polymerization) or using chemical synthesis (e.g., by phosphoramidite chemistry).

[0070] As used herein, the terms "amplify" and "amplification" refer to enzymatically copying the sequence of a polynucleotide, in whole or in part, so as to generate more polynucleotides that also contain the sequence or a complement thereof. The sequence being copied is referred to as the template sequence. Examples of amplification include DNA-templated RNA synthesis by RNA polymerase, RNA-templated first-strand cDNA synthesis by reverse transcriptase, and DNA-templated PCR amplification using a thermostable DNA polymerase. Amplification includes all primer-extension reactions. Amplification includes methods such as PCR, ligation amplification (or ligase chain reaction, LCR) and amplification methods. These methods are known and widely practiced in the art. See, e.g., U.S. Pat. Nos. 4,683,195 and 4,683,202 and Innis et al., "PCR protocols: a guide to method and applications" Academic Press, Incorporated (1990) (for PCR); and Wu et al. (1989) Genomics 4:560-569 (for LCR). In general, the PCR procedure describes a method of gene amplification which is comprised of (i) sequence-specific hybridization of primers to specific genes within a DNA sample (or library), (ii) subsequent amplification involving multiple rounds of annealing, elongation, and denaturation using a DNA polymerase, and (iii) screening the PCR products for a band of the correct size. The primers used are oligonucleotides of sufficient length and appropriate sequence to provide initiation of polymerization, i.e. each primer is specifically designed to be complementary to each strand of the genomic locus to be amplified.

[0071] Reagents and hardware for conducting amplification reaction are commercially available. Primers useful to amplify sequences from a particular gene region are preferably complementary to, and hybridize specifically to sequences in the target region or in its flanking regions and can be prepared using the polynucleotide sequences provided herein. Nucleic acid sequences generated by amplification can be sequenced directly.

[0072] The term "associated" is used herein to refer to the relationship between a sample and the DNA molecules, RNA molecules, or other polynucleotides originating from or derived from that sample. A polynucleotide is associated with a sample if it is an endogenous polynucleotide, i.e., it occurs in the sample at the time the sample is selected or is derived from an endogenous polynucleotide. For example, the mRNAs endogenous to a cell are associated with that cell. cDNAs resulting from reverse transcription of these mRNAs, and DNA amplicons resulting from PCR amplification of the cDNAs, contain the sequences of the mRNAs and are also associated with the cell. The polynucleotides associated with a sample need not be located or synthesized in the sample and are considered associated with the sample even after the sample has been destroyed (for example, after a cell has been lysed). Molecular barcoding or other techniques can be used to determine which polynucleotides in a mixture are associated with a particular sample.

[0073] When hybridization occurs in an antiparallel configuration between two single-stranded polynucleotides, the reaction is called "annealing" and those polynucleotides are described as "complementary". As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of a polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with a polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions can include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C. or 70° C. for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as can be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0074] Complementary sequences include base-pairing of a region of a polynucleotide comprising a first nucleotide sequence to a region of a polynucleotide comprising a second nucleotide sequence over the length or a portion of the length of one or both nucleotide sequences. Such sequences can be referred to as "complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be complementary, or they can include one or more, but generally not more than about 5, 4, 3, or 2 mismatched base pairs within regions that are base-paired. For two sequences with mismatched base pairs, the sequences will be considered "substantially complementary" as long as the two nucleotide sequences bind to each other via base-pairing.

[0075] Conventional notation is used herein to describe nucleotide sequences: the left-hand end of a single-stranded nucleotide sequence is the 5'-end; the left-hand direction of a double-stranded nucleotide sequence is referred to as the 5'-direction. The direction of 5' to 3' addition of nucleotides to nascent RNA transcripts is referred to as the transcription direction. The DNA strand having the same sequence as an mRNA is referred to as the "coding strand"; sequences on the DNA strand having the same sequence as an mRNA

transcribed from that DNA and which are located 5' to the 5'-end of the RNA transcript are referred to as "upstream sequences"; sequences on the DNA strand having the same sequence as the RNA and which are 3' to the 3' end of the coding RNA transcript are referred to as "downstream sequences."

[0076] In some embodiments, the double stranded DNA fragments can be end polished so that they are amenable for ligation. For example, the ends of the DNA fragments can be polished to have blunt ends. As known in the art, this can be achieved with enzymes that can either fill in or remove the protruding strand. Another method is to perform the ligation in the presence of short synthetic oligonucleotides, called "adaptors," which have been prepared in such a way as to eventually ligate with one terminus to the fragment and make the fragment amenable for ligation with polynucleotides of interest such as DNA or RNA tags. As such, the DNA fragments can be ligated to DNA tags.

[0077] In some embodiments, the RNA fragments are end polished by polyadenylation. The RNA fragments can be attached to RNA tags, e.g., on both ends, by template switching reverse transcription.

[0078] A "DNA tag" or "DNA tag molecule" is a polynucleotide comprising a DNA identifier and a UMI. A DNA tag can be a deoxyribopolynucleotide. A "DNA identifier" is a polynucleotide sequence assigned to distinguish a gDNA molecule from a RNA molecule. A DNA tag can be ligated to the 5' or 3' end of double stranded DNA fragments.

[0079] A "RNA tag" or "RNA tag molecule" is a polynucleotide comprising a RNA identifier and a UMI. A RNA tag can be a deoxyribopolynucleotide. A "RNA identifier" is a polynucleotide sequence assigned to distinguish a cDNA molecule from a gDNA molecule. A RNA tag can further comprise poly(T). Alternatively, a RNA tag can further comprise a template switching oligonucleotide (TSO). A RNA tag can be used to add a 5' tag to RNA-derived cDNA fragments through reverse transcription. In some embodiments, a RNA tag can be used to add a 3' tag to RNA-derived cDNA through template switching in reverse transcription.

[0080] Two types of RNA tags are helpful because in order to sequence the single stranded RNA from both directions, two different mechanisms can be used to attach the RNA specific sequence. Only one type of DNA tag is needed because the DNA tag can be ligated to both ends of the double stranded DNA.

[0081] A composition can comprise at least 2 of the tags described above, e.g., a DNA tag and a RNA tag. A composition can also comprise the 3 tags described above, e.g., a DNA tag and the 2 types of RNA tags.

[0082] Unique molecular indices or identifiers (UMIs; also called Random Molecular Tags (RMTs)) are short sequences or "barcodes" of bases used to tag each DNA or RNA molecule (fragment) prior to library amplification, thereby aiding in the identification of each individual nucleic acid molecule, or PCR duplicates. Kivioja, T. et al., *Nat. Methods* 9:72-74 (2012), and Suppl. If two reads align to the same location and have the same UMI, it is highly likely that they are PCR duplicates originating from the same fragment prior to amplification. UMIs can also be used to detect and quantify unique mRNA transcripts. In some embodiments, DNA tags containing the same DNA identifier sequence contain different UMI sequences. In some embodiments, RNA tags containing the same RNA identifier sequence contain different UMI sequences.

[0083] The concept of UMIs is that prior to any amplification, each original target molecule is 'tagged' by a unique barcode sequence. This DNA sequence must be long enough to provide sufficient permutations to assign each founder molecule a unique barcode. In some embodiments, a UMI sequence contains randomized nucleotides and is incorporated into the DNA or RNA tag. For example, a 12-base random sequence provides 4^{12} or 16,777,216 UMI's for each target molecule in the sample.

[0084] In some embodiments, the RNA tag is a single-stranded DNA molecule and serves as a primer for reverse transcription. The RNA tag can be generated using a DNA polymerase (DNAP). Here, the binding site of the RNA tag is an RNA binding site (e.g., an mRNA binding site) and contains a sequence region complementary to a sequence region in one or more RNAs. In some embodiments, the binding site is complementary to a sequence region common to all RNAs in the sample to which the barcode adapter is added. For example, the binding site can be a poly(T) tract, which is complementary to the poly(A) tails of eukaryotic mRNAs. Alternatively, or in addition, the binding site can include a random sequence tract. Upon adding the RNA tag to the RNAs associated with a sample, reverse transcription can occur and first strands of cDNA can be synthesized, such that the RNA identifier sequence is incorporated into the first strands of cDNA. It will be recognized that reverse transcription requires appropriate conditions, for example the presence of an appropriate buffer and reverse transcriptase enzyme, and temperatures appropriate for annealing of the barcode adapter to RNAs and the activity of the enzyme. It will also be recognized that reverse transcription, involving a DNA primer and an RNA template, is most efficient when the 3' end of the primer is complementary to the template and can anneal directly to the template. Accordingly, the RNA tag can be designed so that the binding site occurs at the 3' end of the adapter molecule.

[0085] As described above, the present methods can employ a reverse transcriptase enzyme that adds one or more non-templated nucleotides (such as Cs) to the end of a nascent cDNA strand upon reaching the 5' end of the template RNA. These nucleotides form a 3' DNA overhang at one end of the RNA/DNA duplex. If a second RNA molecule contains a sequence region, for example, a poly-G tract at its 3' end that is complementary to the non-templated nucleotides, and binds to the non-templated nucleotides, the reverse transcriptase can switch templates and continue extending the cDNA, now using the second RNA molecule as a template. Such a second RNA molecule is referred to herein and known in the art as a template-switching oligo (TSO).

[0086] In embodiments of the present methods, a second RNA tag comprising a RNA identifier, UMI, and TSO can serve as a template-switching oligonucleotide for reverse transcription. Thus, the RNA identifier sequence is incorporated into the first strand of cDNA after template switching and is present in DNA molecules resulting from amplification (for example, by PCR) of the first strand of cDNA. In these embodiments, any reverse transcriptase that has template switching activity can be used. The binding site of the first RNA tag is a cDNA binding site and preferably occurs at the 3' end of the adapter molecule. The binding site can include a G-tract (comprising one or more G nucleotides), or any other sequence that is at least partially complementary to that of the 3' overhang generated by the reverse tran-

scriptase. It will be recognized that the overhang sequence, and thus an appropriate sequence for the binding site of the barcode adapter, can depend on the choice of reverse transcriptase used in the method.

[0087] Methods for reverse transcription and template switching are well known in the art. A procedure frequently referred to as "SMART" (switching mechanism at the 5' end of the RNA transcript) can generate full-length cDNA libraries, even from single-cell-derived RNA samples. This strategy relies on the intrinsic properties of Moloney murine leukemia virus (MMLV) reverse transcriptase and the use of a unique template switching oligonucleotide (TS oligo, or TSO). Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) is an RNA-dependent DNA polymerase that can be used in cDNA synthesis with long messenger RNA templates (>5 kb). The enzyme is a product of the pol gene of M-MLV and consists of a single subunit with a molecular weight of 71 kDa. During first-strand synthesis, upon reaching the 5' end of the RNA template, the terminal transferase activity of the MMLV reverse transcriptase adds a few additional nucleotides (mostly deoxycytidine) to the 3' end of the newly synthesized cDNA strand. These bases function as a TS oligo-anchoring site. Upon base pairing between the TS oligo and the appended deoxycytidine stretch, the reverse transcriptase "switches" template strands, from cellular RNA to the TS oligo, and continues replication to the 5' end of the TS oligo. By doing so, the resulting cDNA contains the complete 5' end of the transcript, and universal sequences of choice can be added to the reverse transcription product. Along with tagging of the cDNA 3' end by oligo dT primers, this approach makes it possible to efficiently amplify the entire full-length transcript pool in a completely sequence-independent manner.

[0088] A TS oligo can be a DNA oligo sequence that carries 3 riboguanosines (rGrGrG) at its 3' end. The complementarity between these consecutive rG bases and the 3' dC extension of the cDNA molecule allows the subsequent template switching. The 3' most rG can also be replaced with a locked nucleic acid base (LNA) to enhance thermostability of the LNA monomer, which would be advantageous for base pairing.

[0089] The TSO can include a 3' portion comprising a plurality of guanosines or guanosine analogues that base pair with cytosine. Non-limiting examples of guanosines or guanosine analogues useful in the methods described herein include, but are not limited to, deoxyriboguanosine, riboguanosine, locked nucleic acid-guanosine, and peptide nucleic acid-guanosine. The guanosines can be ribonucleosides or locked nucleic acid monomers.

[0090] The TSO can include a 3' portion including at least 2, at least 3, at least 4, at least 5, or 2, 3, 4, or 5, or 2-5 guanosines, or guanosine analogues that base pair with cytosine. The presence of a plurality of guanosines (or guanosine analogues that base pair with cytosine) allows the TSO to anneal transiently to the exposed cytosines at the 3' end of the first strand of cDNA. This causes the reverse transcriptase to switch template and continue to synthesis a strand complementary to the TSO. In one aspect of the invention, the 3' end of the TSO can be blocked, for example by a 3' phosphate group, to prevent the TSO from functioning as a primer during cDNA synthesis.

[0091] Before the tagged cDNA samples are pooled, synthesis of cDNA can be stopped, for example by removing or inactivating the reverse transcriptase. This prevents cDNA synthesis by reverse transcription from continuing in the pooled samples.

[0092] As used herein, "amplified target sequences" and its derivatives, refers generally to a nucleic acid sequence produced by the amplification of/amplifying the target sequences using target-specific primers and the methods provided herein. The amplified target sequences can be either of the same sense (the positive strand produced in the second round and subsequent even-numbered rounds of amplification) or antisense (i.e., the negative strand produced during the first and subsequent odd-numbered rounds of amplification) with respect to the target sequences. For the purposes of this disclosure, the amplified target sequences are typically less than 50% complementary to any portion of another amplified target sequence in the reaction.

[0093] The term "polymerase chain reaction" ("PCR") of Mullis (U.S. Pat. Nos. 4,683,195, 4,683,202, and 4,965,188) refers to a method for increasing the concentration of a segment of a target sequence in a mixture of nucleic acid sequences without cloning or purification. This process for amplifying the target sequence consists of introducing a large excess of two oligonucleotide primers to the nucleic acid sequence mixture containing the desired target sequence, followed by a precise sequence of thermal cycling in the presence of a polymerase (e.g., DNA polymerase). The two primers are complementary to their respective strands of the double stranded target sequence. To effect amplification, the mixture is denatured and the primers then annealed to their complementary sequences within the target molecule. Following annealing, the primers are extended with a polymerase so as to form a new pair of complementary strands. The steps of denaturation, primer annealing, and polymerase extension can be repeated many times (i.e., denaturation, annealing and extension constitute one "cycle;" there can be numerous "cycles") to obtain a high concentration of an amplified segment of the desired target sequence. The length of the amplified segment of the desired target sequence is determined by the relative positions of the primers with respect to each other, and therefore, this length is a controllable parameter. By virtue of the repeating aspect of the process, the method is referred to as the "polymerase chain reaction" (hereinafter "PCR"). Because the desired amplified segments of the target sequence become the predominant sequences (in terms of concentration) in the mixture, they are said to be "PCR amplified."

[0094] The methods disclosed herein can further comprise amplifying the tagged DNA the tagged cDNA for enrichment with a set of gene specific primers. Target enrichment can be achieved with, e.g., an SPE primer pool, DNA boosting primer, and RNA boosting primer. Amplicon-based next-generation sequencing (NGS) assays offer many advantages for targeted enrichment. For example, QIAseq NGS panels employ unique molecular indices (UMI's) to correct for PCR amplification bias and use single primer extension (SPE) technology which provides design flexibility and highly-specific target enrichment. The concept of UMIs is that prior to any amplification, each original target molecule is 'tagged' by a unique barcode sequence. This DNA sequence must be long enough to provide sufficient permutations to assign each founder molecule a unique barcode. In its current form, a 12-base random sequence provides 4^{12} or 16,777,216 UMI's for each target molecule in the sample.

[0095] As used herein, the term "primer" includes an oligonucleotide, either natural or synthetic, that is capable, upon forming a duplex with a polynucleotide template, of acting as a point of initiation of nucleic acid synthesis and being extended from its 3' end along the template so that an extended duplex is formed. The sequence of nucleotides added during the extension process is determined by the sequence of the template polynucleotide. Usually primers are extended by a DNA polymerase. Primers usually have a length in the range of between 3 to 36 nucleotides, also 5 to 24 nucleotides, also from 14 to 36 nucleotides. Primers within the scope of the invention include orthogonal primers, amplification primers, constructions primers and the like. Pairs of primers can flank a sequence of interest or a set of sequences of interest. Primers and probes can be degenerate in sequence. Primers within the scope of the present invention bind adjacent to a target sequence. A "primer" can be considered a short polynucleotide, generally with a free 3'-OH group that binds to a target or template potentially present in a sample of interest by hybridizing with the target, and thereafter promoting polymerization of a polynucleotide complementary to the target. Primers of the instant invention are comprised of nucleotides ranging from 17 to 30 nucleotides. In some embodiments, the primer is at least 17 nucleotides, or alternatively, at least 18 nucleotides, or alternatively, at least 19 nucleotides, or alternatively, at least 20 nucleotides, or alternatively, at least 21 nucleotides, or alternatively, at least 22 nucleotides, or alternatively, at least 23 nucleotides, or alternatively, at least 24 nucleotides, or alternatively, at least 25 nucleotides, or alternatively, at least 26 nucleotides, or alternatively, at least 27 nucleotides, or alternatively, at least 28 nucleotides, or alternatively, at least 29 nucleotides, or alternatively, at least 30 nucleotides, or alternatively at least 50 nucleotides, or alternatively at least 75 nucleotides or alternatively at least 100 nucleotides.

[0096] As used herein, "target-specific primer" and its derivatives, refers generally to a single stranded or double-stranded polynucleotide, typically an oligonucleotide, that includes at least one sequence that is at least 50% complementary, typically at least 75% complementary or at least 85% complementary, more typically at least 90% complementary, more typically at least 95% complementary, more typically at least 98% or at least 99% complementary, or 100% identical, to at least a portion of a nucleic acid molecule that includes a target sequence. In such instances, the target-specific primer and target sequence are described as "corresponding" to each other. In some embodiments, the target-specific primer is capable of hybridizing to at least a portion of its corresponding target sequence (or to a complement of the target sequence); such hybridization can optionally be performed under standard hybridization conditions or under stringent hybridization conditions. In some embodiments, the target-specific primer is not capable of hybridizing to the target sequence, or to its complement, but is capable of hybridizing to a portion of a nucleic acid strand including the target sequence, or to its complement. In some embodiments, the target-specific primer includes at least one sequence that is at least 75% complementary, typically at least 85% complementary, more typically at least 90% complementary, more typically at least 95% complementary, more typically at least 98% complementary, or more typically at least 99% complementary, to at least a portion of the target sequence itself; in other embodiments, the target-specific primer includes at least one sequence that is at least 75% complementary, typically at least 85% complementary, more typically at least 90% complementary, more typically at least 95% complementary, more typically at least 98% complementary, or more typically at least 99% complementary, to at least a portion of the nucleic acid molecule other than the target sequence. In some embodiments, the target-specific primer is substantially non-complementary to other target sequences present in the sample; optionally, the target-specific primer is substantially non-complementary to other nucleic acid molecules present in the sample. In some embodiments, nucleic acid molecules present in the sample that do not include or correspond to a target sequence (or to a complement of the target sequence) are referred to as "non-specific" sequences or "non-specific nucleic acids". In some embodiments, the target-specific primer is designed to include a nucleotide sequence that is substantially complementary to at least a portion of its corresponding target sequence. In some embodiments, a target-specific primer is at least 95% complementary, or at least 99% complementary, or 100% identical, across its entire length to at least a portion of a nucleic acid molecule that includes its corresponding target sequence. In some embodiments, a target-specific primer can be at least 90%, at least 95% complementary, at least 98% complementary or at least 99% complementary, or 100% identical, across its entire length to at least a portion of its corresponding target sequence. In some embodiments, a forward target-specific primer and a reverse target-specific primer define a target-specific primer pair that can be used to amplify the target sequence via template-dependent primer extension. Typically, each primer of a target-specific primer pair includes at least one sequence that is substantially complementary to at least a portion of a nucleic acid molecule including a corresponding target sequence but that is less than 50% complementary to at least one other target sequence in the sample. In some embodiments, amplification can be performed using multiple target-specific primer pairs in a single amplification reaction, wherein each primer pair includes a forward target-specific primer and a reverse target-specific primer, each including at least one sequence that substantially complementary or substantially identical to a corresponding target sequence in the sample, and each primer pair having a different corresponding target sequence. In some embodiments, the target-specific primer can be substantially non-complementary at its 3' end or its 5' end to any other target-specific primer present in an amplification reaction. In some embodiments, the target-specific primer can include minimal cross hybridization to other target-specific primers in the amplification reaction. In some embodiments, target-specific primers include minimal cross-hybridization to non-specific sequences in the amplification reaction mixture. In some embodiments, the target-specific primers include minimal self-complementarity. In some embodiments, the target-specific primers can include one or more cleavable groups located at the 3' end. In some embodiments, the target-specific primers can include one or more cleavable groups located near or about a central nucleotide of the target-specific primer. In some embodiments, one of more targets-specific primers includes only non-cleavable nucleotides at the 5' end of the target-specific primer. In some embodiments, a target specific primer includes minimal nucleotide sequence overlap at the 3'end or the 5' end of the primer as compared to one or more different target-specific primers, optionally in the same amplification reaction. In some

embodiments 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more, target-specific primers in a single reaction mixture include one or more of the above embodiments. In some embodiments, substantially all of the plurality of target-specific primers in a single reaction mixture includes one or more of the above embodiments.

[0097] Primer design is based on single primer extension, in which each genomic target is enriched by one target-specific primer and one universal primer—a strategy that removes conventional two target-specific primer design restriction and reduces the amount of required primers. All primers required for a panel are pooled into an individual primer pool to reduce panel handling and the number of pools required for enrichment and library construction.

[0098] The booster panel is a pool of up to 100 primers that can be used to boost the performance of certain primers in any panel (cataloged, extended, or custom), or to extend the contents of an existing custom panel. The primers are delivered as a single pool that can be spiked into the existing panel.

[0099] After removing unused adapters, a limited number of PCR cycles can be conducted using an adapter primer and a pool of single primers, each carrying a gene specific sequence and a 5' universal sequence. During this process, each single primer repeatedly samples the same target locus from different DNA templates. Afterwards, additional PCR cycles can be conducted using universal primers to attach complete adapter sequences and to amplify the library to the desired quantity.

[0100] Compared to existing targeted enrichment approaches, the SPE method relies on single end adapter ligation, which inherently has a much higher efficiency than requiring adapters to ligate to both ends of the dsDNA fragment. More DNA molecules will be available for the downstream PCR enrichment step. PCR enrichment efficiency using one primer is also better than conventional two primer approach, due to the absence of an efficiency constraint from a second primer. During the initial PCR cycles, primers have repeated opportunities to convert (i.e. capture) maximal amount of original DNA molecules into amplicons.

[0101] All three features help to increase the efficiency of capturing rare mutations in the sample. In addition, incorporated UMI's within the amplicon are the key to estimating the number of DNA molecules captured and to greatly reduce sequencing errors in downstream analysis. Single primer extension also permits discovery of unknown structural variants, such as gene fusions.

[0102] The targeted enriched sample of DNA (e.g., gDNA) and cDNA are split into 2 separate samples. A first sample can be amplified by polymerase chain reaction (PCR) using primers specific for the DNA tag to generate a DNA library corresponding to the DNA in the sample. A second sample can be amplified by PCR using primers specific for the RNA tag to generate a cDNA library corresponding to the RNA in the sample.

[0103] A real-time polymerase chain reaction (Real-Time PCR), also known as quantitative polymerase chain reaction (qPCR), is a laboratory technique of molecular biology based on the polymerase chain reaction (PCR). It monitors the amplification of a targeted DNA molecule during the PCR, i.e. in real-time, and not at its end, as in conventional PCR. Real-time PCR can be used quantitatively (quantitative real-time PCR), and semi-quantitatively, i.e. above/below a certain amount of DNA molecules (semi quantitative real-time PCR). Other types of PCRs include but are not limited to nested PCR (used to analyze DNA sequences coming from different organisms of the same species but that can differ for a single nucleotide (SNIPS) and to ensure amplification of the sequence of interest in each of the organism analyzed) and Inverse-PCR (usually used to clone a region flanking an insert or a transposable element).

[0104] Two common methods for the detection of PCR products in real-time PCR are: (1) non-specific fluorescent dyes that intercalate with any double-stranded DNA, and (2) sequence-specific DNA probes consisting of oligonucleotides that are labeled with a fluorescent reporter which permits detection only after hybridization of the probe with its complementary sequence.

[0105] Methods and kits for performing PCR are well known in the art. PCR is a reaction in which replicate copies are made of a target polynucleotide using a pair of primers or a set of primers consisting of an upstream and a downstream primer, and a catalyst of polymerization, such as a DNA polymerase, and typically a thermally-stable polymerase enzyme. Methods for PCR are well known in the art, and taught, for example in MacPherson et al. (1991) PCR 1: A Practical Approach (IRL Press at Oxford University Press).

[0106] Embodiments of the invention provide 2 separate libraries for flexible manipulation downstream: a DNA library based on the original DNA and a cDNA library based on the original RNA produced by any of the methods described herein. The DNA library or cDNA library can be sequenced to provide an analysis of gene expression in single cells or in a plurality of single cells.

[0107] The amplified DNA or cDNA library can be sequenced and analyzed using methods known to those of skill in the art, e.g., by next-generation sequencing (NGS). In certain exemplary embodiments, RNA expression profiles are determined using any sequencing methods known in the art. Determination of the sequence of a nucleic acid sequence of interest can be performed using a variety of sequencing methods known in the art including, but not limited to, sequencing by synthesis (SBS), sequencing by hybridization (SBH), sequencing by ligation (SBL) (Shendure et al. (2005) Science 309:1728), quantitative incremental fluorescent nucleotide addition sequencing (QIFNAS), stepwise ligation and cleavage, fluorescence resonance energy transfer (FRET), molecular beacons, TaqMan reporter probe digestion, pyrosequencing, fluorescent in situ sequencing (FISSEQ), FISSEQ beads (U.S. Pat. No. 7,425,431), wobble sequencing (PCT/US05/27695), multiplex sequencing (U.S. Ser. No. 12/027,039, filed Feb. 6, 2008; Porreca et al (2007) Nat. Methods 4:931), polymerized colony (POLONY) sequencing (U.S. Pat. Nos. 6,432,360, 6,485,944 and 6,511,803, and PCT/US05/06425); nanogrid rolling circle sequencing (ROLONY) (US2009/0018024), allele-specific oligo ligation assays (e.g., oligo ligation assay (OLA), single template molecule OLA using a ligated linear probe and a rolling circle amplification (RCA) readout, ligated padlock probes, and/or single template molecule OLA using a ligated circular padlock probe and a rolling circle amplification (RCA) readout) and the like. High-throughput sequencing methods, e.g., using platforms such as Roche 454, Illumina Solexa, AB-SOLiD, Helicos, Complete Genomics, Polonator platforms and the like, can also be utilized. A variety of light-based sequencing technologies are known in the art (Landegren et al. (1998) Genome Res.

8:769-76; Kwok (2000) Pharmacogenomics 1:95-100; and Shi (2001) Clin. Chem. 47:164-172).

[0108] Embodiments of the invention also provide methods for analyzing gene expression in a plurality of single cells, the method comprising the steps of preparing a cDNA library using the method described herein and sequencing the cDNA library. A "gene" refers to a polynucleotide containing at least one open reading frame (ORF) that is capable of encoding a particular polypeptide or protein after being transcribed and translated. Any of the polynucleotide sequences described herein can be used to identify larger fragments or full-length coding sequences of the gene with which they are associated. Methods of isolating larger fragment sequences are known to those of skill in the art.

[0109] As used herein, "expression" refers to the process by which polynucleotides are transcribed into mRNA and/or the process by which the transcribed mRNA is subsequently being translated into peptides, polypeptides, or proteins. If the polynucleotide is derived from genomic DNA, expression can include splicing of the mRNA in a eukaryotic cell.

[0110] The cDNA library can be sequenced by any suitable screening method. In particular, the cDNA library can be sequenced using a high-throughput screening method, such as Applied Biosystems' SOLiD sequencing technology, or Illumina's Genome Analyzer. In one aspect of the invention, the cDNA library can be shotgun sequenced. The number of reads can be at least 10,000, at least 1 million, at least 10 million, at least 100 million, or at least 1000 million. In another aspect, the number of reads can be from 10,000 to 100,000, or alternatively from 100,000 to 1 million, or alternatively from 1 million to 10 million, or alternatively from 10 million to 100 million, or alternatively from 100 million to 1000 million. A "read" is a length of continuous nucleic acid sequence obtained by a sequencing reaction.

[0111] The DNA or gDNA library generated by the methods disclosed herein can be useful for, but not limited to, DNA variant detection, copy number analysis, fusion gene detection and structural variant detection. The cDNA library generated by the methods disclosed herein can be useful for, but not limited to, RNA variant detection, gene expression analysis, and fusion gene detection. The DNA and cDNA libraries can also be used for paired DNA and RNA profiling.

[0112] The expression profiles described herein are useful in the field of predictive medicine in which diagnostic assays, prognostic assays, pharmacogenomics, and monitoring clinical trials are used for prognostic (predictive) purposes to thereby treat an individual prophylactically. Accordingly, some embodiments relate to diagnostic assays for determining the expression profile of nucleic acid sequences (e.g., RNAs), in order to determine whether an individual is at risk of developing a disorder and/or disease. Such assays can be used for prognostic or predictive purposes to thereby prophylactically treat an individual prior to the onset of the disorder and/or disease. Accordingly, in certain exemplary embodiments, methods of diagnosing and/or prognosing one or more diseases and/or disorders using one or more of expression profiling methods described herein are provided.

[0113] Some embodiments pertain to monitoring the influence of agents (e.g., drugs or other compounds administered either to inhibit or to treat or prevent a disorder and/or disease) on the expression profile of nucleic acid sequences (e.g., RNAs) in clinical trials. Accordingly, in certain exemplary embodiments, methods of monitoring one or more diseases and/or disorders before, during and/or subsequent to treatment with one or more agents using one or more of expression profiling methods described herein are provided.

[0114] Monitoring the influence of agents (e.g., drug compounds) on the level of expression of a marker of the invention can be applied not only in basic drug screening, but also in clinical trials. For example, the effectiveness of an agent to affect an expression profile can be monitored in clinical trials of subjects receiving treatment for a disease and/or disorder associated with the expression profile. In certain exemplary embodiments, the methods for monitoring the effectiveness of treatment of a subject with an agent (e.g., an agonist, antagonist, peptidomimetic, protein, peptide, nucleic acid, small molecule, or other drug candidate) comprising the steps of (i) obtaining a pre-administration sample from a subject prior to administration of the agent; (ii) detecting one or more expression profiled in the pre-administration sample; (iii) obtaining one or more post-administration samples from the subject; (iv) detecting one or more expression profiles in the post-administration samples; (v) comparing the one or more expression profiled in the pre-administration sample with the one or more expression profiles in the post-administration sample or samples; and (vi) altering the administration of the agent to the subject accordingly.

[0115] The expression profiling methods described herein allow the quantitation of gene expression. Thus, not only tissue specificity, but also the level of expression of a variety of genes in the tissue is ascertainable. Thus, genes can be grouped on the basis of their tissue expression per se and level of expression in that tissue. This is useful, for example, in ascertaining the relationship of gene expression between or among tissues. Thus, one tissue can be perturbed and the effect on gene expression in a second tissue can be determined. In this context, the effect of one cell type on another cell type in response to a biological stimulus can be determined. Such a determination is useful, for example, to know the effect of cell-cell interaction at the level of gene expression. If an agent is administered therapeutically to treat one cell type but has an undesirable effect on another cell type, the invention provides an assay to determine the molecular basis of the undesirable effect and thus provides the opportunity to co-administer a counteracting agent or otherwise treat the undesired effect. Similarly, even within a single cell type, undesirable biological effects can be determined at the molecular level. Thus, the effects of an agent on expression of other than the target gene can be ascertained and counteracted.

[0116] In another embodiment, the time course of expression of one or more nucleic acid sequences (e.g., genes, mRNAs and the like) in an expression profile can be monitored. This can occur in various biological contexts, as disclosed herein, for example development of a disease and/or disorder, progression of a disease and/or disorder, and processes, such as cellular alterations associated with the disease and/or disorder.

[0117] The expression profiling methods described herein are also useful for ascertaining the effect of the expression of one or more nucleic acid sequences (e.g., genes, mRNAs and the like) on the expression of other nucleic acid sequences (e.g., genes, mRNAs and the like) in the same cell or in different cells. This provides, for example, for a

selection of alternate molecular targets for therapeutic intervention if the ultimate or downstream target cannot be regulated.

[0118] The expression profiling methods described herein are also useful for ascertaining differential expression patterns of one or more nucleic acid sequences (e.g., genes, mRNAs and the like) in normal and abnormal cells. This provides a battery of nucleic acid sequences (e.g., genes, mRNAs and the like) that could serve as a molecular target for diagnosis or therapeutic intervention.

EXAMPLES

[0119] Starting Material: Purified genomic DNA and total RNA. For example, 50 ng gDNA and 50 ng total RNA was purified from THP-1 cell line. Ideally, the relative amount of gDNA and RNA should represent the content in the sample.

[0120] DNA/RNA Fragmentation:

	uL	final conc.
DNA/RNA sample	X	
H_2O	11.8 − x	
10× Fragmentation Buffer	2	1×
100 mM dATP	0.6	3 mM
Exonuclease I (20 U/uL)	1.6	1.6 U/uL
5× Fragmentation Enzyme Mix	4	1×
	Total volume: 20 uL	
Incubate in thermocycler with heated lid on for 4° C. 1 min → 32° C. 15 min → 75° C. 10 min → 80° C. 20 min → 4° C. hold		

[0121] RNA Polyadenylation:

	uL	final conc.
Sample from previous step	20	
H_2O	0.5	
10 mM ATP	1.25	0.5 mM
10 mM 3'-dATP (blocker)	1.25	0.5 mM
T4 Polynucleotide Kinase (10 U/uL)	1	0.4 U/uL
E. coli Poly(A) Polymerase (5 U/uL)	1	0.2 U/uL
	Total volume: 25 uL	
Incubate in thermocycler with heated lid on for 4° C. 1 min → 30° C. 10 min → 4° C. hold		

[0122] DNA Ligation:

	uL	final conc.
Sample from previous step	25	
5× Ligation Buffer	10	1×
50 uM DNA ligation Adaptor	2.8	2.8 uM
50% PEG-6000	7.2	7.2%
T4 DNA ligase (600 U/uL)	5	60 U/uL
	Total volume: 50 uL	
Incubate in thermocycler with heated lid OFF for 4° C. 1 min → 20° C. 15 min → 4° C. hold		

[0123] Purification: Add 50 uL of ice cold water to the 50 uL sample from previous step to make 100 uL total. Do 2 rounds of 1.2× Ampure XP beads purification following manufacturer's manual with the following exceptions: 1st round elution in 52 uL water; and 2nd round elution in 13 uL water.

[0124] Reverse Transcription:

	uL	final conc.
Sample from previous step	12.87	
7.5 uM TSON10T18NV oligo	1	300 nM
25 uM TSON10forTS oligo	1	1 uM
5× SuperScript II Buffer	5	1×
25 mM each dNTP mix	1	1 mM each
0.1M DTT	1.25	5 mM
RNase Inhibitor (40 U/uL)	0.63	1 U/uL
300 mM $MgCl_2$	0.5	6 mM
150 mM $MnCl_2$	0.5	3 mM
MMLV Reverse Trancriptase RNase H− (200 U/uL)	1.25	10 U/uL
	Total volume: 25 uL	
Incubate in thermocycler with heated lid on for 4° C. 1 min → 25° C. 10 min → 42° C. 45 min → 70° C. 15 min → 4° C. hold		

[0125] Purification: Add 75 uL of ice cold water to the 25 uL sample from previous step to make 100 uL total. Do 1 round of 1.2× Ampure XP beads purification following manufacturer's manual and elute in 16.8 uL water.

[0126] Target Enrichment:

	uL	final conc.
Sample from previous step	16.8	
5× V2 Buffer	8	1×
2 mM each dNTP mix	4	0.2 mM each
100 nM each SPE primer pool	8	20 nM each
10 uM DNA boosting primer	0.8	400 nM
10 uM RNA boosting primer	0.8	400 nM
Hot-Star Taq Polymerase (6 U/uL)	1.6	0.24 U/uL
	Total volume: 40 uL	
Incubate in thermocycler with heated lid on for 95° C. 13 min → 98° C. 2 min → 8 cycles of (98° C. 15 sec → 68° C. 10 min) → 72° C. 5 min → 4° C. hold		

[0127] Purification: Add 60 uL of ice cold water to the 40 uL sample from previous step to make 100 uL total. Do double size selection 0.5×/0.5× with Ampure XP beads following manufacturer's manual and elute in 22 uL water.

[0128] qPCR (real-time) to determine final amplification cycles:

	For DNA library		For RNA library	
	uL	final conc.	uL	final conc.
Sample from previous step	2		2	
5× V2 Buffer	2	1×	2	1×
2 mM each dNTP mix	1	0.2 mM each	1	0.2 mM each
H_2O	2.1		2.1	
20× EveGreen Dye	0.5	1×	0.5	1×
4 uM IL2N5RS2 Universal primer	1	400 nM	1	400 nM
4 uM DNA Universal Primer	1	400 nM	0	0
4 uM RNA Universal Primer	0	0	1	400 nM
Hot-Star Taq Polymerase (6 U/uL)	0.4	0.24 U/uL	0.4	0.24 U/uL
	Total volume: 10 uL		Total volume: 10 uL	
Run on ABI 7900 real time instrument: 95° C. 13 min → 98° C. 2 min → 30 cycles of (98° C. 15 sec → 62° C. 2 min). Record the counts for both samples				

[0129] Universal PCR:

	For DNA library		For RNA library	
	uL	final conc.	uL	final conc.
Sample from Target Enrichment	9		9	
5× V2 Buffer	5	1×	5	1×
2 mM each dNTP mix	2.5	0.2 mM each	2.5	0.2 mM each
4 uM IL2N5RS2 Universal primer	2.5	400 nM	2.5	400 nM
4 uM DNA Universal Primer	2.5	400 nM	0	0
4 uM RNA Universal Primer	0	0	2.5	400 nM
H_2O	2.5		2.5	
Hot-Star Taq Polymerase (6 U/uL)	1	0.24 U/uL	1	0.24 U/uL
	Total volume: 25 uL		Total volume: 25 uL	
Incubate in thermocycler with heated lid on for 95° C. 13 min → 98° C. 2 min → "X" cycles of (98° C. 15 sec → 62° C. 2 min) → 72° C. 5 min → 4° C. hold (X = Ct + 4) for DNA sample and RNA sample respectively. For example, if Ct = 19 for DNA, and 15 for RNA, then run 23 cycles for DNA, and 19 cycles for RNA				

[0130] Purification: Add 75 uL of ice cold water to each of the 25 uL sample from previous step to make 100 uL total. Do 1 round of 1.2× Ampure XP beads purification following manufacturer's manual and elute in 20 uL water.

[0131] Library Quantification using Agilent Bioanalyzer High Sensitivity DNA chip: Dilute the purified libraries to 2 ng/uL. Load 1 uL of this diluted sample on the bioanalyzer. Obtain molar concentration of the libraries based on bioanalyzer's electropherogram. The libraries are ready for sequencing.

[0132] Following the workflow, with 50 ng gDNA and 50 ng total RNA input, we obtained 675 ng of DNA library and 455 ng of RNA library. The same amount of 50 ng total RNA was also used with QIAseq Targeted RNAscan Panels system from QIAGEN for comparison purpose. The same amount of 50 ng gDNA was also used with QIAseq Targeted DNA Panels system from QIAGEN for comparison purpose. The samples were then put on Illumina's MiSeq machine for sequencing.

Results

[0133] As shown in Table 1, compared to the standalone RNA library prep workflow (QIAseq Targeted RNAscan Panels system from QIAGEN), our method achieved around 24% of its enrichment efficiency on the 1^{st} strand cDNA, and around 40% of its enrichment efficiency on the 2^{nd} strand cDNA. Since RNAscan workflow had strand bias toward the 1^{st} strand, our method had less bias and improved strand balance. The effect of enrichment efficiency on RNA analysis deserves further exploration.

TABLE 1

Workflow	RNAScan	Ours
Average UMIs/ primer 1^{st} strand	11061	2681
Average UMIs/ primer 2^{nd} strand	5279	2077
Ratio $2^{nd}/1^{st}$	0.48	0.77

[0134] UMI per SPE primer for RNA sample: Primers were divided into two groups based on the RNA strand they detected. As shown in Table 2, compared to the standalone DNA library prep workflow (QIAseq Targeted DNA Panels system from QIAGEN), our method achieved slightly better enrichment efficiency. Both of the methods had comparable sequencing specificity and uniformity.

TABLE 2

Workflow	Targeted DNA Panels	Ours
Average UMIs/primer	1471	1701
Average reads/UMI	3.4	3.0
Overall specificity (on-target reads/all reads)	87%	90%
Coverage uniformity (T50)	24.9	21.6

[0135] Sequencing specs for DNA sample in both methods: Sequence coverage uniformity was measured by T50, the percentage of total sequence throughput captured by the bottom 50% of a target region. In the perfect uniform scenario, the T50 value equals to 50.

[0136] Cross talk between DNA and RNA was also evaluated since they remained in the same reaction. Using the same 50 ng of DNA and RNA from THP-1 cell line, the effective leaking signal from RNA to DNA was only 0.75% of the real DNA signal, as measured by the total UMIs of the primers detecting both RNA and DNA. In this case, only the extremely highly expressed genes might have an effect on corresponding DNA copy number analysis. However, if DNA copy number analysis was limited on intron regions, this effect should disappear. The effective leaking signal from DNA to RNA was around 3% on average by the same measurement. Since there were only a few copies of genome DNA in each cell in most cases, this kind of leaking could only affect those extremely low expressing genes (less than 0.1 copy per cell), which might be lower than the background noise level. In conclusion, our method demonstrated minimal cross talk between DNA and RNA samples which might not have any significant effect in real cases.

[0137] The DNA library prepared by our method can be used for DNA variant detection, and copy number analysis. The RNA library prepared by our method is suitable for gene expression analysis, fusion gene detection, and RNA variant detection. Multi-modal NGS panels can be developed based on our proposed method, and be used for biomarker screening, or targeted eQTL analysis.

[0138] Adaptor for ligation:

Equal molar mix and annealing of the following 2 oligos to make double strand adaptor (DNA ligation Adaptor)		
SEQ ID NO:1	/5Phos/GGACTCCAATNNNNNNNNNNNNACGCTAA GAAAGATCGGAAGAGCACACGTCTG/3ddC/	PAGE Purified
SEQ ID NO:2	ATT+GGAG+TCC*T/3Phos/	STD desalt
Reverse Transcription Oligos:		
SEQ ID NO:3 TSON10T18 NV oligo	CGACTCACTATAGGGCTGGAATTCTGACGNNNNNNN NNNACGTTTTTTTTTTTTTTTTTNV	PAGE Purified
SEQ ID NO:4 TSON10for TS oligo	/5Me-isodC//iisodG//iisodG/TAATACGACTCACTATAG GGCTGGAATTCTGACGNNNNNNNNNNATCTGCrGrGrG	PAGE Purified

Target Enrichment Oligos:		
SEQ ID NO: 5 DNA boosting primer	AGCAGTGGTATCAACGCAGAGTCAAGC AGAAGACGGCATACGAGATTCCGAAAC GTGACTGGAGTTCAGACGTGTGCTCTT CCGATCTTTCTTAGCGT	STD desalt
SEQ ID NO: 6 RNA boosting primer	GTGAGTGATGGTTGAGGATGTGTGCAA GCAGAAGACGGCATACGAGATTACGTA CGGTGACTGGAGTTCAGACGTGTGCTC TTCCGATCTCGACTCACTATAGGGCTG GAATTCT	STD desalt

For each primer, the first set of underlined nucleotides is priming site for PCR amplification in Universal PCR reactions, the second set of underlined nucleotides in the middle is the sample idx (index) region, which can be replaced with respective sample index sequences, and the third set of underlined nucleotides is part of DNA or RNA identifier used for PCR amplification in target enrichment reactions.

[0139] uPCR Primers:

PAGE Purified
IL2N5RS2 Universal primer

SEQ ID NO: 7
AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTC

TTCCGATCTNNNNNNAATGTACAGTATTGCGTTTTG

STD desalt
DNA Universal Primer

SEQ ID NO: 8
AAGCAGTGGTATCAACGCAGAGT

STD desalt
RNA Universal Primer

SEQ ID NO: 9
GTGAGTGATGGTTGAGGATGTGTG

[0140] SPE Primer Pool (equal molar mix of the following oligos):

SEQ ID NO: 10
AATGTACAGTATTGCGTTTTGAGCCCCAAGTCCTATGAGAACCTCTG

SEQ ID NO: 11
AATGTACAGTATTGCGTTTTGTGGCACCAGCGATCAGGTCCTTTAT

SEQ ID NO: 12
AATGTACAGTATTGCGTTTTGCTGAGTGGAGTCACAGCGGAGATAGT

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SEQ ID NO: 13
AATGTACAGTATTGCGTTTTGTGTTCCACCAGTAACAACAGTTGAATGT

CC

SEQ ID NO: 14
AATGTACAGTATTGCGTTTTGGTGTGAGGAACATACTAGTGCTTTGCAA

GT

SEQ ID NO: 15
AATGTACAGTATTGCGTTTTGTTCAAAGTTGGGTCTGCTTCAGTCCAAAG

SEQ ID NO: 16
AATGTACAGTATTGCGTTTTGCCCCCAGCTTCTTCTCTCTGCACTAAG

SEQ ID NO: 17
AATGTACAGTATTGCGTTTTGGCCTTCCCAACATGCATTCTAACTTCTT

CC

SEQ ID NO: 18
AATGTACAGTATTGCGTTTTGCCAGCTACTCTCAAAATCAGCATCCTTT

GG

SEQ ID NO: 19
AATGTACAGTATTGCGTTTTGCCAGTCCTTCTGTGAGTCTATCCTCAGT

TC

SEQ ID NO: 20
AATGTACAGTATTGCGTTTTGAGAGCGAACCAAGAATGCCTGTTTACAG

SEQ ID NO: 21
AATGTACAGTATTGCGTTTTGGAGAGGCACGAGAACACACATCTATTCTG

SEQ ID NO: 22
AATGTACAGTATTGCGTTTTGTTCTCTTCAGAAGTTCCTTCGTCATCCTT

SEQ ID NO: 23
AATGTACAGTATTGCGTTTTGTGATGACATGCCCCATCACTAAAACAC

SEQ ID NO: 24
AATGTACAGTATTGCGTTTTGTGATAGAGACATGATGTAACCGTGGGAAT

TTCTTC

SEQ ID NO: 25
AATGTACAGTATTGCGTTTTGCGTTCTAAGAGAGTGACAGAAAGGTAAAG

AGGAG

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SEQ ID NO: 26
AATGTACAGTATTGCGTTTTGATCACAAAGTATCTTTTTCTGTGGCTTAG

AAATCTT

SEQ ID NO: 27
AATGTACAGTATTGCGTTTTGTCAAATGTTAGCTCATTTTTGTTAATGGT

GGCTTTT

SEQ ID NO: 28
AATGTACAGTATTGCGTTTTGTGTCACATTATAAAGATTCAGGCAATGTT

TGTTAGT

SEQ ID NO: 29
AATGTACAGTATTGCGTTTTGAGTTTGTATGCAACATTTCTAAAGTTACC

TACTTGT

SEQ ID NO: 30
AATGTACAGTATTGCGTTTTGAAAATCTGTTTTCCAATAAATTCTCAGAT

CCAGGAA

SEQ ID NO: 31
AATGTACAGTATTGCGTTTTGCGACCCAGTTACCATAGCAATTTAGTGAA

ATAACTA

SEQ ID NO: 32
AATGTACAGTATTGCGTTTTGAGAGGCGCTATGTGTATTATTATAGCTAC

CTGTTAA

SEQ ID NO: 33
AATGTACAGTATTGCGTTTTGCGTTTTTGACAGTTTGACAGTTAAAGGCA

TTTCC

SEQ ID NO: 34
AATGTACAGTATTGCGTTTTGCTGTCCTTATTTTGGATATTTCTCCCAAT

GAAAGTA

SEQ ID NO: 35
AATGTACAGTATTGCGTTTTGGACTTTTTGCAAATGTTTAACATAGGTGA

CAGATTT

SEQ ID NO: 36
AATGTACAGTATTGCGTTTTGAAGTAGAAAATGGAAGTCTATGTGATCAA

GAAATCG

SEQ ID NO: 37
AATGTACAGTATTGCGTTTTGGGCCTCTTAAAGATCATGTTTGTTACAGT

GCTTA

SEQ ID NO: 38
AATGTACAGTATTGCGTTTTGACAAGATTGGTCAGGAAAAGAGAATTGTT

CCTATAA

SEQ ID NO: 39
AATGTACAGTATTGCGTTTTGAGACCCTGTCTCAAAAGTAAAAAGTAAGT

TAACATG

SEQ ID NO: 40
AATGTACAGTATTGCGTTTTGTCAGTGTCTTCCAAATCCTTATGTATAGC

AGCAAT

SEQ ID NO: 41
AATGTACAGTATTGCGTTTTGAGGGTCGAGGAAGCCAGTTTACATCAA

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SEQ ID NO: 42
AATGTACAGTATTGCGTTTTGAACAAAAAGATATTTTCAATATTTCTGCG

CAGGTTT

SEQ ID NO: 43
AATGTACAGTATTGCGTTTTGGTCTCGACTTGAATTGCAAAAAGATGTTA

GAAAAGC

SEQ ID NO: 44
AATGTACAGTATTGCGTTTTGAAAATGTTGGCAGTCATAACATTTGAAAC

TAATGGA

SEQ ID NO: 45
AATGTACAGTATTGCGTTTTGAGCCTCAAACAGGTTGGTTTTAAATTTGA

AGTCT

SEQ ID NO: 46
AATGTACAGTATTGCGTTTTGCCTCTGTGTGTATGTTTTAACTACAAAGC

GAAACA

SEQ ID NO: 47
AATGTACAGTATTGCGTTTTGGATTCACCTGGTAATGAGGAAAACAGCTT

TAAAATC

SEQ ID NO: 48
AATGTACAGTATTGCGTTTTGAGATCTGCTGAAAAGAAATTTGTTAAAGC

ACAATT

SEQ ID NO: 49
AATGTACAGTATTGCGTTTTGCGGCATCCCCTACATCGAGACCTC

SEQ ID NO: 50
AATGTACAGTATTGCGTTTTGCAGGGAGCAGATCAAACGGGTGAAG

SEQ ID NO: 51
AATGTACAGTATTGCGTTTTGCAAGTCTTTTGAGGACATCCACCAGTAC

AG

SEQ ID NO: 52
AATGTACAGTATTGCGTTTTGACGTGCCTGTTGGACATCCTGGATA

SEQ ID NO: 53
AATGTACAGTATTGCGTTTTGCCTGTACTGGTGGATGTCCTCAAAAGACT

SEQ ID NO: 54
AATGTACAGTATTGCGTTTTGCCCTGAGGAGCGATGACGGAATATAAGC

SEQ ID NO: 55
AATGTACAGTATTGCGTTTTGGTCGTATTCGTCCACAAAATGGTTCTGGA

TC

SEQ ID NO: 56
AATGTACAGTATTGCGTTTTGTGACTGGCAATTGTGTCAACAGGTGAAAA

SEQ ID NO: 57
AATGTACAGTATTGCGTTTTGCGCCAGCTGGAGTTTGGTCATGTTT

SEQ ID NO: 58
AATGTACAGTATTGCGTTTTGAATCCCTCTCATCACAATTTCATTCCACA

ATAGTTT

SEQ ID NO: 59
AATGTACAGTATTGCGTTTTGTCAACAACAAAGAGAATCATGAAATCAAC

CCTAGC

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SEQ ID NO: 60
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SEQ ID NO: 61
AATGTACAGTATTGCGTTTTGGGCGCGGAAAGTCCTCACTCTC

SEQ ID NO: 62
AATGTACAGTATTGCGTTTTGTATGGTGAGGTTCGGCGTGTTTAAACG

SEQ ID NO: 63
AATGTACAGTATTGCGTTTTGTGGTGACAAAGTTAGAAGGGTCCATGG

SEQ ID NO: 64
AATGTACAGTATTGCGTTTTGCTTCTTTACCACCCCAGATACGACGACTA

SEQ ID NO: 65
AATGTACAGTATTGCGTTTTGCGCTCGTGGTGGTAGTCGTCGTAT

SEQ ID NO: 66
AATGTACAGTATTGCGTTTTGCCAGGAGGCCCTTTCTGTTTACAACC

SEQ ID NO: 67
AATGTACAGTATTGCGTTTTGCCCACAAGCCCAAAATATTCTACTCACTT

TGC

SEQ ID NO: 68
AATGTACAGTATTGCGTTTTGATCGCCTGCATCAAGGAAAAGGTAATGG

SEQ ID NO: 69
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SEQ ID NO: 70
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SEQ ID NO: 71
AATGTACAGTATTGCGTTTTGGGAAATGCTCTCACGTAGTCTCTCATGT

CT

SEQ ID NO: 72
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TA

SEQ ID NO: 73
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SEQ ID NO: 74
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SEQ ID NO: 75
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TTC

SEQ ID NO: 76
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SEQ ID NO: 77
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TAAACT

SEQ ID NO: 78
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CC

SEQ ID NO: 79
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TATCCTA

SEQ ID NO: 81
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TC

SEQ ID NO: 82
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SEQ ID NO: 83
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AGCGTTA

SEQ ID NO: 84
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TGCATTT

SEQ ID NO: 85
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SEQ ID NO: 86
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CCGAATT

SEQ ID NO: 87
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TCATC

SEQ ID NO: 88
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TAC

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SEQ ID NO: 90
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CTC

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AT

SEQ ID NO: 92
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SEQ ID NO: 93
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TTCCAAG

SEQ ID NO: 94
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CT

SEQ ID NO: 95
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TT

SEQ ID NO: 96
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SEQ ID NO: 100
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SEQ ID NO: 101
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SEQ ID NO: 102
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GTTG

SEQ ID NO: 103
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SEQ ID NO: 104
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SEQ ID NO: 105
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TCCTC

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SEQ ID NO: 107
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SEQ ID NO: 108
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SEQ ID NO: 109
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SEQ ID NO: 110
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SEQ ID NO: 111
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CAAC

SEQ ID NO: 112
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SEQ ID NO: 113
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AC

SEQ ID NO: 114
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TG

SEQ ID NO: 115
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SEQ ID NO: 116
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SEQ ID NO: 117
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SEQ ID NO: 119
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SEQ ID NO: 120
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SEQ ID NO: 121
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SEQ ID NO: 122
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AG

SEQ ID NO: 123
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SEQ ID NO: 124
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SEQ ID NO: 125
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CACTTCT

SEQ ID NO: 126
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ATGAC

SEQ ID NO: 127
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SEQ ID NO: 128
AATGTACAGTATTGCGTTTTGAGGCTGGTACCTGCTCTTCTTCAATC

SEQ ID NO: 129
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GTC

SEQ ID NO: 130
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GGAAA

SEQ ID NO: 131
AATGTACAGTATTGCGTTTTGTCCAGGATAATACACATCACAGTAAATAA

CACTCTG

SEQ ID NO: 132
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TTTGA

SEQ ID NO: 133
AATGTACAGTATTGCGTTTTGCTCCCATTTTTGTGCATCTTTGTTGCTG

TC

SEQ ID NO: 134
AATGTACAGTATTGCGTTTTGCAGAACTGCCTATTCCTAACTGACTCATC

ATTTC

SEQ ID NO: 135
AATGTACAGTATTGCGTTTTGGAATTCTGTTTCATCGCTGAGTGACACTC

TTTT

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SEQ ID NO: 136
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SEQ ID NO: 137
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GAA

SEQ ID NO: 138
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SEQ ID NO: 139
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TCTTCCA

SEQ ID NO: 140
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SEQ ID NO: 141
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ATCAAGG

SEQ ID NO: 142
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TCTACCC

SEQ ID NO: 143
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CTCTT

SEQ ID NO: 144
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AAGAGGA

SEQ ID NO: 146
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SEQ ID NO: 149
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SEQ ID NO: 153
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SEQ ID NO: 154
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SEQ ID NO: 159
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SEQ ID NO: 165
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SEQ ID NO: 166
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AA

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SEQ ID NO: 178
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ATC

SEQ ID NO: 271
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GT

SEQ ID NO: 272
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SEQ ID NO: 313
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TC

SEQ ID NO: 383
AATGTACAGTATTGCGTTTTGAATCGAAGCGCTACCTGATTCCAATTCC

SEQ ID NO: 384
AATGTACAGTATTGCGTTTTGCCGACCGTAACTATTCGGTGCGTTG

-continued

SEQ ID NO: 385
AATGTACAGTATTGCGTTTTGACATTCTATCCAAGCTGTGTTCTATCTTG

AGAAACT

SEQ ID NO: 386
AATGTACAGTATTGCGTTTTGCGAGTGAGGGTTTTCGTGGTTCACATC

SEQ ID NO: 387
AATGTACAGTATTGCGTTTTGCGTGGGTCCCAGTCTGCAGTTAAG

SEQ ID NO: 388
AATGTACAGTATTGCGTTTTGGCTCAGAGCCGTTCCGAGATCTT

SEQ ID NO: 389
AATGTACAGTATTGCGTTTTGGCGTTCCATCTCCCACTTGTCGTAGTT

SEQ ID NO: 390
AATGTACAGTATTGCGTTTTGCTGGCCGAGTTGGTTCATCATCATTCAA

SEQ ID NO: 391
AATGTACAGTATTGCGTTTTGTATGGTGTGTCCCCCAACTACGACAAG

SEQ ID NO: 392
AATGTACAGTATTGCGTTTTGTGAAAAGCACTTCCTGAAATAATTTCACC

TTCGTTT

SEQ ID NO: 393
AATGTACAGTATTGCGTTTTGAGGTACTCCATGGCTGACGAGATCTG

SEQ ID NO: 394
AATGTACAGTATTGCGTTTTGTTGCCTTTGTTCCAAGGTCCAATGTGT

SEQ ID NO: 395
AATGTACAGTATTGCGTTTTGCGTCCCCGCATTCCAACGTCTC

SEQ ID NO: 396
AATGTACAGTATTGCGTTTTGGGCGCGCCGTTTACTTGAAGG

SEQ ID NO: 397
AATGTACAGTATTGCGTTTTGGCCTGGCGGTGCACACTATTCTG

SEQ ID NO: 398
AATGTACAGTATTGCGTTTTGAGGTGCAGCCACAAAACTTACAGATGC

SEQ ID NO: 399
AATGTACAGTATTGCGTTTTGGTGCCGAACCAATACAACCCTCTG

SEQ ID NO: 400
AATGTACAGTATTGCGTTTTGGGGCGGGTCCACCAGTTTGAAT

SEQ ID NO: 401
AATGTACAGTATTGCGTTTTGCCGCAGAGGGTTGTATTGGTTCG

SEQ ID NO: 402
AATGTACAGTATTGCGTTTTGAGCCACTCGCATTGACCATTCAAACT

SEQ ID NO: 403
AATGTACAGTATTGCGTTTTGCCACGTCTGACAGGTAGCCATGG

SEQ ID NO: 404
AATGTACAGTATTGCGTTTTGGTGAGGCTGCTGGACGAGTACAAC

SEQ ID NO: 405
AATGTACAGTATTGCGTTTTGCGCACCAGGTTGTACTCGTCCA

SEQ ID NO: 406
AATGTACAGTATTGCGTTTTGCCGCCTTTGTGCTTCTGTTCTTCGT

SEQ ID NO: 407
AATGTACAGTATTGCGTTTTGCTGATTAATCGCGTAGAAAATGACCTTAT

TTTGGAG

SEQ ID NO: 408
AATGTACAGTATTGCGTTTTGGCTCCATCGTCTACCTGGAGATTGACAA

-continued

SEQ ID NO: 409
AATGTACAGTATTGCGTTTTGTCTGCACGGCCTCGATCTTGTAGG

SEQ ID NO: 410
AATGTACAGTATTGCGTTTTGGCCAGCAGATGATCTTCCCCTACTACG

SEQ ID NO: 411
AATGTACAGTATTGCGTTTTGCGTCACGCTTGAAGACCACGTTG

SEQ ID NO: 412
AATGTACAGTATTGCGTTTTGGCCAGCATGCAGTTCTAAGGCTCT

SEQ ID NO: 413
AATGTACAGTATTGCGTTTTGGTGCCCGTCTCGACTCTTAGGC

SEQ ID NO: 414
AATGTACAGTATTGCGTTTTGTGTAGCCGCTGATCGTCGTGTATATGTC

SEQ ID NO: 415
AATGTACAGTATTGCGTTTTGGACTGGTACTGGTTAGTAAAGGTTGATAA

TATTCCA

SEQ ID NO: 416
AATGTACAGTATTGCGTTTTGGGTGAAGTAATCAGTTTGTTCACTAGTTA

CGTGATT

SEQ ID NO: 417
AATGTACAGTATTGCGTTTTGCTGACATGCCTACTGATTATTCTTCAAAC

TCATCAC

SEQ ID NO: 418
AATGTACAGTATTGCGTTTTGTGTGTGTTTTAATTGTTCCACTTGAGATT

CTTAACC

SEQ ID NO: 419
AATGTACAGTATTGCGTTTTGCGTCAGCATTTTGAATCACTTCATTCTGA

CATGATA

SEQ ID NO: 420
AATGTACAGTATTGCGTTTTGAGTAATTTTCAACTATTGGCCTAGTGAAT

TTAAGCT

SEQ ID NO: 421
AATGTACAGTATTGCGTTTTGAGAAAGAGGGAAGTCACATTTATAGAGTG

CTAGC

SEQ ID NO: 422
AATGTACAGTATTGCGTTTTGCATCAACAGAAACAGAACAACAAACTGTG

ACAAATC

SEQ ID NO: 423
AATGTACAGTATTGCGTTTTGCCAAAGAATATCCCTTTATATAGCAGTGG

AACAATT

SEQ ID NO: 424
AATGTACAGTATTGCGTTTTGCAGAATATGCAGTGATAAGTGCTGTTTCA

TCACT

SEQ ID NO: 425
AATGTACAGTATTGCGTTTTGTTCCCCCTGTGACGACTACTTTTCCTC

SEQ ID NO: 426
AATGTACAGTATTGCGTTTTGCGGTCCCTATTTCTTCCTCTGCTTCGT

SEQ ID NO: 427
AATGTACAGTATTGCGTTTTGCTGAACAGTTCTGTCTCTATTACCCGACC

TC

-continued

SEQ ID NO: 428
AATGTACAGTATTGCGTTTTGCGTTCATAGCCTTCTATCCGAGTATGTAG

CA

SEQ ID NO: 429
AATGTACAGTATTGCGTTTTGCCCCTTCTGTCCTCGCAGGTTAATCC

SEQ ID NO: 430
AATGTACAGTATTGCGTTTTGGCTTCCAGCCATTTCTGAGATATCCTCAC

AGT

SEQ ID NO: 431
AATGTACAGTATTGCGTTTTGACCAGGAGGAACAAAGACACATGAAGATC

AT

SEQ ID NO: 432
AATGTACAGTATTGCGTTTTGGCGCCCCCGAGTTTCTTACGAATC

SEQ ID NO: 433
AATGTACAGTATTGCGTTTTGTTTATACACAGTTTGGAGTTTGAGAATCA

GAAGACT

SEQ ID NO: 434
AATGTACAGTATTGCGTTTTGGGTTATCTCTGGCTGATGAGATTATGAGT

GATTCTC

SEQ ID NO: 435
AATGTACAGTATTGCGTTTTGGCCAAGCTAGTGATTGATGTGATTCGCT

AT

SEQ ID NO: 436
AATGTACAGTATTGCGTTTTGCCCCTCCTCTAGTACTCCCTGTTTGT

SEQ ID NO: 437
AATGTACAGTATTGCGTTTTGCTCCTTCCTGTCCCAATCAACTAGTCTA

GC

SEQ ID NO: 438
AATGTACAGTATTGCGTTTTGGCCTCGTCCCTCTTCCCTTAGGTAA

SEQ ID NO: 439
AATGTACAGTATTGCGTTTTGTCTCTCTTCCCATTAGTCTGAGTACTGAG

TGATT

SEQ ID NO: 440
AATGTACAGTATTGCGTTTTGAGCATTTCTTGAGACTTAAAGTGGCATTC

TAAAGG

SEQ ID NO: 441
AATGTACAGTATTGCGTTTTGATTTTTATTCTCAAGAGGCAGAAATACCA

ACTTACC

SEQ ID NO: 442
AATGTACAGTATTGCGTTTTGAATTTATAGCTCTTTTCATCTGCTTTGGT

ATCATCA

SEQ ID NO: 443
AATGTACAGTATTGCGTTTTGGCCTCTAATCTGATATACAGCCTTAGAAA

GTCACA

SEQ ID NO: 444
AATGTACAGTATTGCGTTTTGTGTGCCATTGTCCTGGAGCAACAATT

-continued

SEQ ID NO: 445
AATGTACAGTATTGCGTTTTGAGTGTACTGCTCGTTTTCTTAATTTGAAA

AGTGAGT

SEQ ID NO: 446
AATGTACAGTATTGCGTTTTGACCCATGAACTAATACTTATTTTGAGATT

GGTCCAT

SEQ ID NO: 447
AATGTACAGTATTGCGTTTTGCATGGTGCAACAAAAGTAAGAATCCAACA

GTTTT

SEQ ID NO: 448
AATGTACAGTATTGCGTTTTGTTGAAATGTTAAGTAAGCTTGAAATACCG

ATAGCAT

SEQ ID NO: 449
AATGTACAGTATTGCGTTTTGGGGAGGAAGAAAATGAAGCACGAGGAAA

AC

SEQ ID NO: 450
AATGTACAGTATTGCGTTTTGATTTGGGATGTACTCTAAATTTAAAGCAG

CAAATCA

SEQ ID NO: 451
AATGTACAGTATTGCGTTTTGTCAAGAGCAGAATTTGGAGACTTTGATAT

TAAAACT

SEQ ID NO: 452
AATGTACAGTATTGCGTTTTGCGGTTACTAACATGTTTAGGGAAATAGAC

AACTGTT

-continued

SEQ ID NO: 453
AATGTACAGTATTGCGTTTTGCCTGACAACAGATCCCATATAATTAACTT

TCATACC

SEQ ID NO: 454
AATGTACAGTATTGCGTTTTGAGATGAAGAAGATGAGGAACGAGAGAGTA

AAAGC

[0141] The foregoing description of the specific embodiments will so fully reveal the general nature of the invention that others can, by applying knowledge within the skill of the art, readily modify and/or adapt for various applications, without departing from the general concept of the invention. Therefore, such adaptations and modifications are intended to be within the meaning and range of equivalents of the disclosed embodiments, based on the teaching and guidance presented herein. It is to be understood that the phraseology or terminology herein is for the purpose of description and not of limitation, such that the terminology or phraseology of the present specification is to be interpreted by the skilled artisan in light of the teachings and guidance.

[0142] The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments but should be defined only in accordance with the following claims and their equivalents.

[0143] All of the various aspects, embodiments, and options described herein can be combined in any and all variations.

[0144] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be herein incorporated by reference. U.S. Appl. No. 62/648,174, filed Mar. 26, 2018, is incorporated herein by reference in its entirety.

SEQUENCE LISTING

Sequence total quantity: 454
SEQ ID NO: 1 moltype = DNA length = 55
FEATURE Location/Qualifiers
misc_feature 1..55
note = Adaptor
misc_difference 11..22
note = n is a or c or g or t
modified_base 55
mod_base = OTHER
note = c is dideoxycytosine
source 1..55
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 1
ggactccaat nnnnnnnnnn nnacgctaag aaagatcgga agagcacacg tctgc 55

SEQ ID NO: 2 moltype = DNA length = 11
FEATURE Location/Qualifiers
misc_feature 1..11
note = adaptor
source 1..11
mol_type = other DNA
organism = synthetic construct
SEQUENCE: 2
attggagtcc t 11

SEQ ID NO: 3 moltype = DNA length = 62
FEATURE Location/Qualifiers
misc_feature 1..62
note = Oligonucleotide

-continued

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misc_difference        30..39
                       note = n is a or c or g or t
misc_difference        61
                       note = n is a or c or g or t
misc_difference        62
                       note = v is a or c or g
source                 1..62
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 3
cgactcacta tagggctgga attctgacgn nnnnnnnnna cgtttttttt ttttttttt  60
nv                                                                62

SEQ ID NO: 4           moltype = DNA  length = 56
FEATURE                Location/Qualifiers
source                 1..56
                       mol_type = other DNA
                       organism = synthetic construct
misc_feature           1..56
                       note = Oligonucleotide
variation              38..47
                       note = n is a or c or g or t
modified_base          1
                       mod_base = OTHER
                       note = c is 5-methyldeoxyisocytidine
modified_base          54..56
                       mod_base = OTHER
                       note = g is riboguanosine
modified_base          2..3
                       mod_base = OTHER
                       note = g is deoxyisoguanosine
SEQUENCE: 4
cggtaatacg actcactata gggctggaat tctgacgnnn nnnnnnnatc tgcggg      56

SEQ ID NO: 5           moltype = DNA  length = 98
FEATURE                Location/Qualifiers
misc_feature           1..98
                       note = Primer
source                 1..98
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 5
agcagtggta tcaacgcaga gtcaagcaga agacggcata cgagattccg aaacgtgact  60
ggagttcaga cgtgtgctct tccgatcttt cttagcgt                          98

SEQ ID NO: 6           moltype = DNA  length = 115
FEATURE                Location/Qualifiers
misc_feature           1..115
                       note = Primer
source                 1..115
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 6
gtgagtgatg gttgaggatg tgtgcaagca gaagacggca tacgagatta cgtacggtga  60
ctggagttca gacgtgtgct cttccgatct cgactcacta tagggctgga attct       115

SEQ ID NO: 7           moltype = DNA  length = 84
FEATURE                Location/Qualifiers
misc_feature           1..84
                       note = Primer
misc_difference        59..63
                       note = n is a or c or g or t
source                 1..84
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 7
aatgatacgg cgaccaccga gatctacact ctttccctac acgacgctct tccgatctnn  60
nnnaatgtac agtattgcgt tttg                                         84

SEQ ID NO: 8           moltype = DNA  length = 23
FEATURE                Location/Qualifiers
misc_feature           1..23
                       note = Primer
source                 1..23
                       mol_type = other DNA
                       organism = synthetic construct
```

-continued

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SEQUENCE: 8
aagcagtggt atcaacgcag agt                                          23

SEQ ID NO: 9          moltype = DNA  length = 24
FEATURE               Location/Qualifiers
misc_feature          1..24
                      note = Primer
source                1..24
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 9
gtgagtgatg gttgaggatg tgtg                                         24

SEQ ID NO: 10         moltype = DNA  length = 47
FEATURE               Location/Qualifiers
misc_feature          1..47
                      note = primer
source                1..47
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 10
aatgtacagt attgcgtttt gagccccaag tcctatgaga acctctg                47

SEQ ID NO: 11         moltype = DNA  length = 46
FEATURE               Location/Qualifiers
misc_feature          1..46
                      note = primer
source                1..46
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 11
aatgtacagt attgcgtttt gtggcaccag cgatcaggtc ctttat                 46

SEQ ID NO: 12         moltype = DNA  length = 47
FEATURE               Location/Qualifiers
misc_feature          1..47
                      note = Primer
source                1..47
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 12
aatgtacagt attgcgtttt gctgagtgga gtcacagcgg agatagt                47

SEQ ID NO: 13         moltype = DNA  length = 51
FEATURE               Location/Qualifiers
misc_feature          1..51
                      note = Primer
source                1..51
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 13
aatgtacagt attgcgtttt gtgttccacc agtaacaaca gttgaatgtc c            51

SEQ ID NO: 14         moltype = DNA  length = 51
FEATURE               Location/Qualifiers
misc_feature          1..51
                      note = Primer
source                1..51
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 14
aatgtacagt attgcgtttt ggtgtgagga acatactagt gctttgcaag t            51

SEQ ID NO: 15         moltype = DNA  length = 50
FEATURE               Location/Qualifiers
misc_feature          1..50
                      note = Primer
source                1..50
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 15
aatgtacagt attgcgtttt gttcaaagtt gggtctgctt cagtccaaag              50

SEQ ID NO: 16         moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
```

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```
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 16
aatgtacagt attgcgtttt gcccccagct tcttctctct gcactaag              48

SEQ ID NO: 17        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 17
aatgtacagt attgcgtttt ggccttccca acatgcattc taacttcttc c           51

SEQ ID NO: 18        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 18
aatgtacagt attgcgtttt gccagctact ctcaaaatca gcatcctttg g           51

SEQ ID NO: 19        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 19
aatgtacagt attgcgtttt gccagtcctt ctgtgagtct atcctcagtt c           51

SEQ ID NO: 20        moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 20
aatgtacagt attgcgtttt gagagcgaac caagaatgcc tgtttacag              49

SEQ ID NO: 21        moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 21
aatgtacagt attgcgtttt ggagaggcac gagaacacac atctattctg             50

SEQ ID NO: 22        moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 22
aatgtacagt attgcgtttt gttctcttca gaagttcctt cgtcatcctt             50

SEQ ID NO: 23        moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 23
aatgtacagt attgcgtttt gtgatgacat gccccatcac taaaacac               48
```

-continued

```
SEQ ID NO: 24          moltype = DNA  length = 56
FEATURE                Location/Qualifiers
misc_feature           1..56
                       note = Primer
source                 1..56
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 24
aatgtacagt attgcgtttt gtgatagaga catgatgtaa ccgtgggaat ttcttc      56

SEQ ID NO: 25          moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 25
aatgtacagt attgcgtttt gcgttctaag agagtgacag aaaggtaaag aggag       55

SEQ ID NO: 26          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 26
aatgtacagt attgcgtttt gatcacaaag tatctttttc tgtggcttag aaatctt     57

SEQ ID NO: 27          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 27
aatgtacagt attgcgtttt gtcaaatgtt agctcatttt tgttaatggt ggctttt     57

SEQ ID NO: 28          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 28
aatgtacagt attgcgtttt gtgtcacatt ataaagattc aggcaatgtt tgttagt     57

SEQ ID NO: 29          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 29
aatgtacagt attgcgtttt gagtttgtat gcaacatttc taaagttacc tacttgt     57

SEQ ID NO: 30          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 30
aatgtacagt attgcgtttt gaaaatctgt tttccaataa attctcagat ccaggaa     57

SEQ ID NO: 31          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
```

-continued

```
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 31
aatgtacagt attgcgtttt gcgacccagt taccatagca atttagtgaa ataacta     57

SEQ ID NO: 32        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 32
aatgtacagt attgcgtttt gagaggcgct atgtgtatta ttatagctac ctgttaa     57

SEQ ID NO: 33        moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 33
aatgtacagt attgcgtttt gcgtttttga cagtttgaca gttaaaggca tttcc       55

SEQ ID NO: 34        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 34
aatgtacagt attgcgtttt gctgtcctta ttttggatat ttctcccaat gaaagta     57

SEQ ID NO: 35        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 35
aatgtacagt attgcgtttt ggacttttttg caaatgttta acataggtga cagattt     57

SEQ ID NO: 36        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 36
aatgtacagt attgcgtttt gaagtagaaa atggaagtct atgtgatcaa gaaatcg     57

SEQ ID NO: 37        moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 37
aatgtacagt attgcgtttt gggcctctta aagatcatgt ttgttacagt gctta       55

SEQ ID NO: 38        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 38
aatgtacagt attgcgtttt gacaagattg gtcaggaaaa gagaattgtt cctataa     57

SEQ ID NO: 39        moltype = DNA  length = 57
```

-continued

```
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 39
aatgtacagt attgcgtttt gagaccctgt ctcaaaagta aaaagtaagt taacatg     57

SEQ ID NO: 40         moltype = DNA  length = 56
FEATURE               Location/Qualifiers
misc_feature          1..56
                      note = Primer
source                1..56
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 40
aatgtacagt attgcgtttt gtcagtgtct tccaaatcct tatgtatagc agcaat      56

SEQ ID NO: 41         moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 41
aatgtacagt attgcgtttt gagggtcgag gaagccagtt tacatcaa               48

SEQ ID NO: 42         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 42
aatgtacagt attgcgtttt gaacaaaaag atattttcaa tatttctgcg caggttt     57

SEQ ID NO: 43         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 43
aatgtacagt attgcgtttt ggtctcgact tgaattgcaa aaagatgtta gaaaagc     57

SEQ ID NO: 44         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 44
aatgtacagt attgcgtttt gaaaatgttg gcagtcataa catttgaaac taatgga     57

SEQ ID NO: 45         moltype = DNA  length = 55
FEATURE               Location/Qualifiers
misc_feature          1..55
                      note = Primer
source                1..55
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 45
aatgtacagt attgcgtttt gagcctcaaa caggttggtt ttaaatttga agtct       55

SEQ ID NO: 46         moltype = DNA  length = 56
FEATURE               Location/Qualifiers
misc_feature          1..56
                      note = Primer
source                1..56
                      mol_type = other DNA
                      organism = synthetic construct
```

-continued

```
SEQUENCE: 46
aatgtacagt attgcgtttt gcctctgtgt gtatgtttta actacaaagc gaaaca      56

SEQ ID NO: 47          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 47
aatgtacagt attgcgtttt ggattcacct ggtaatgagg aaaacagctt taaaatc     57

SEQ ID NO: 48          moltype = DNA  length = 56
FEATURE                Location/Qualifiers
misc_feature           1..56
                       note = Primer
source                 1..56
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 48
aatgtacagt attgcgtttt gagatctgct gaaaagaaat ttgttaaagc acaatt      56

SEQ ID NO: 49          moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 49
aatgtacagt attgcgtttt gcggcatccc ctacatcgag acctc                  45

SEQ ID NO: 50          moltype = DNA  length = 46
FEATURE                Location/Qualifiers
misc_feature           1..46
                       note = Primer
source                 1..46
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 50
aatgtacagt attgcgtttt gcagggagca gatcaaacgg gtgaag                 46

SEQ ID NO: 51          moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 51
aatgtacagt attgcgtttt gcaagtcttt tgaggacatc caccagtaca g           51

SEQ ID NO: 52          moltype = DNA  length = 46
FEATURE                Location/Qualifiers
misc_feature           1..46
                       note = Primer
source                 1..46
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 52
aatgtacagt attgcgtttt gacgtgcctg ttggacatcc tggata                 46

SEQ ID NO: 53          moltype = DNA  length = 50
FEATURE                Location/Qualifiers
misc_feature           1..50
                       note = Primer
source                 1..50
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 53
aatgtacagt attgcgtttt gcctgtactg gtggatgtcc tcaaaagact             50

SEQ ID NO: 54          moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
```

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                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 54
aatgtacagt attgcgtttt gccctgagga gcgatgacgg aatataagc              49

SEQ ID NO: 55        moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 55
aatgtacagt attgcgtttt ggtcgtattc gtccacaaaa tggttctgga tc          52

SEQ ID NO: 56        moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 56
aatgtacagt attgcgtttt gtgactggca attgtgtcaa caggtgaaaa              50

SEQ ID NO: 57        moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 57
aatgtacagt attgcgtttt gcgccagctg gagtttggtc atgttt                46

SEQ ID NO: 58        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 58
aatgtacagt attgcgtttt gaatccctct catcacaatt tcattccaca atagttt     57

SEQ ID NO: 59        moltype = DNA  length = 56
FEATURE              Location/Qualifiers
misc_feature         1..56
                     note = Primer
source               1..56
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 59
aatgtacagt attgcgtttt gtcaacaaca aagagaatca tgaaatcaac cctagc      56

SEQ ID NO: 60        moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 60
aatgtacagt attgcgtttt ggatatggag ccagcgtgtt ccgatt                46

SEQ ID NO: 61        moltype = DNA  length = 43
FEATURE              Location/Qualifiers
misc_feature         1..43
                     note = Primer
source               1..43
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 61
aatgtacagt attgcgtttt gggcgcggaa agtcctcact ctc                   43
```

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SEQ ID NO: 62          moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 62
aatgtacagt attgcgtttt gtatggtgag gttcggcgtg tttaaacg              48

SEQ ID NO: 63          moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 63
aatgtacagt attgcgtttt gtggtgacaa agttagaagg gtccatgg              48

SEQ ID NO: 64          moltype = DNA  length = 50
FEATURE                Location/Qualifiers
misc_feature           1..50
                       note = Primer
source                 1..50
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 64
aatgtacagt attgcgtttt gcttctttac caccccagat acgacgacta             50

SEQ ID NO: 65          moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 65
aatgtacagt attgcgtttt gcgctcgtgg tggtagtcgt cgtat                  45

SEQ ID NO: 66          moltype = DNA  length = 47
FEATURE                Location/Qualifiers
misc_feature           1..47
                       note = Primer
source                 1..47
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 66
aatgtacagt attgcgtttt gccaggaggc cctttctgtt tacaacc                47

SEQ ID NO: 67          moltype = DNA  length = 53
FEATURE                Location/Qualifiers
misc_feature           1..53
                       note = Primer
source                 1..53
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 67
aatgtacagt attgcgtttt gcccacaagc ccaaaatatt ctactcactt tgc          53

SEQ ID NO: 68          moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 68
aatgtacagt attgcgtttt gatcgcctgc atcaaggaaa aggtaatgg              49

SEQ ID NO: 69          moltype = DNA  length = 50
FEATURE                Location/Qualifiers
misc_feature           1..50
                       note = Primer
source                 1..50
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 69
aatgtacagt attgcgtttt gcgcgtaagg atagcaactg aggttatcac            50

SEQ ID NO: 70        moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 70
aatgtacagt attgcgtttt gcgacctgac gtaacccctt gcttatc                47

SEQ ID NO: 71        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 71
aatgtacagt attgcgtttt gggaaatgct ctcacgtagt ctctcatgtc t           51

SEQ ID NO: 72        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 72
aatgtacagt attgcgtttt ggtcataacc cgaagaacaa tgttgccact a           51

SEQ ID NO: 73        moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 73
aatgtacagt attgcgtttt ggtcagctca ggataaagca cggatggata             50

SEQ ID NO: 74        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 74
aatgtacagt attgcgtttt gctcaggata aaagcttcct tcttaacaag tttttcc     57

SEQ ID NO: 75        moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 75
aatgtacagt attgcgtttt gagagattgt tcccttgcat tgacctcttt ttc         53

SEQ ID NO: 76        moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 76
aatgtacagt attgcgtttt gcccctcacc tttggaattt acagtctgaa             50

SEQ ID NO: 77        moltype = DNA  length = 56
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FEATURE               Location/Qualifiers
misc_feature          1..56
                      note = Primer
source                1..56
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 77
aatgtacagt attgcgtttt gtaggttctt caggtctcta cactctcctt taaact      56

SEQ ID NO: 78         moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 78
aatgtacagt attgcgtttt ggagaaggag tgcaatgcca agattatgat cc          52

SEQ ID NO: 79         moltype = DNA  length = 50
FEATURE               Location/Qualifiers
misc_feature          1..50
                      note = Primer
source                1..50
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 79
aatgtacagt attgcgtttt ggacgttctc cattgtattg gcagtaacca             50

SEQ ID NO: 80         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 80
aatgtacagt attgcgtttt gcacatctca caggctctaa aggaattcta tatccta      57

SEQ ID NO: 81         moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 81
aatgtacagt attgcgtttt ggaggcaaga ggtgagtagt accaatactg tc          52

SEQ ID NO: 82         moltype = DNA  length = 47
FEATURE               Location/Qualifiers
misc_feature          1..47
                      note = Prmer
source                1..47
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 82
aatgtacagt attgcgtttt ggagcccctc cgcttacttg taatctg                47

SEQ ID NO: 83         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 83
aatgtacagt attgcgtttt gccagtaaaa cgtattgaga aaaaggtaaa agcgtta      57

SEQ ID NO: 84         moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 84
aatgtacagt attgcgtttt ggctcagaat aaatcgtaac aatctcaaag tgcattt     57

SEQ ID NO: 85          moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 85
aatgtacagt attgcgtttt gtgaggtgtc cacagggctc aatctttac              49

SEQ ID NO: 86          moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 86
aatgtacagt attgcgtttt gccccttgta tcagtaaagg ctatataata ccgaatt     57

SEQ ID NO: 87          moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 87
aatgtacagt attgcgtttt gtcatgaaga gagtatcatc agctcgttca tcatc       55

SEQ ID NO: 88          moltype = DNA  length = 53
FEATURE                Location/Qualifiers
misc_feature           1..53
                       note = Primer
source                 1..53
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 88
aatgtacagt attgcgtttt gtgtcctttc tgccgatgtg aaattaaagg tac         53

SEQ ID NO: 89          moltype = DNA  length = 47
FEATURE                Location/Qualifiers
misc_feature           1..47
                       note = Primer
source                 1..47
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 89
aatgtacagt attgcgtttt gtcgccccaa ataatttcct gcgaaca                47

SEQ ID NO: 90          moltype = DNA  length = 53
FEATURE                Location/Qualifiers
misc_feature           1..53
                       note = Primer
source                 1..53
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 90
aatgtacagt attgcgtttt gctcatacct ccattccaag ctttcattgt ctc          53

SEQ ID NO: 91          moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 91
aatgtacagt attgcgtttt gcctgccctt atttttaaca gcaggaacga at          52

SEQ ID NO: 92          moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
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                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 92
aatgtacagt attgcgtttt gtcgatagcg aaagtcctct ttggtcag              48

SEQ ID NO: 93        moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 93
aatgtacagt attgcgtttt ggttaaagac caaccactaa ctaagagact ttccaag     57

SEQ ID NO: 94        moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = Primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 94
aatgtacagt attgcgtttt gaaacctctt ccagtacctt cttcatggtt ct          52

SEQ ID NO: 95        moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 95
aatgtacagt attgcgtttt gtttccaggt gatgtgctct atgaactcct t           51

SEQ ID NO: 96        moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 96
aatgtacagt attgcgtttt gggagcggtg caacagttca atggt                  45

SEQ ID NO: 97        moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 97
aatgtacagt attgcgtttt gcatccgtgg ataatgtgca ccataacc               48

SEQ ID NO: 98        moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 98
aatgtacagt attgcgtttt gtcggagagc ctggactgtt tgaaatc                47

SEQ ID NO: 99        moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 99
aatgtacagt attgcgtttt gaagccaggt cttcccgatg agagag                 46
```

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SEQ ID NO: 100          moltype = DNA  length = 46
FEATURE                 Location/Qualifiers
misc_feature            1..46
                        note = Primer
source                  1..46
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 100
aatgtacagt attgcgtttt gggcactccg tggatttcaa acagtc                46

SEQ ID NO: 101          moltype = DNA  length = 53
FEATURE                 Location/Qualifiers
misc_feature            1..53
                        note = Primer
source                  1..53
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 101
aatgtacagt attgcgtttt gcagatatct gctgcccttt taccttatgg ttt         53

SEQ ID NO: 102          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 102
aatgtacagt attgcgtttt gtgtagactg ctttgggatt acgtctatca gttg        54

SEQ ID NO: 103          moltype = DNA  length = 52
FEATURE                 Location/Qualifiers
misc_feature            1..52
                        note = primer
source                  1..52
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 103
aatgtacagt attgcgtttt gggaaaggag aaaaaggaag tgctacctga ac          52

SEQ ID NO: 104          moltype = DNA  length = 51
FEATURE                 Location/Qualifiers
misc_feature            1..51
                        note = Primer
source                  1..51
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 104
aatgtacagt attgcgtttt gtttttctcc cttcctcctt tgaacaaaca g           51

SEQ ID NO: 105          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 105
aatgtacagt attgcgtttt gacagcttta ggaaaatgga atctcttacc tcctc       55

SEQ ID NO: 106          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 106
aatgtacagt attgcgtttt ggggtgttat ggtcgcgttg gatttctg               48

SEQ ID NO: 107          moltype = DNA  length = 45
FEATURE                 Location/Qualifiers
misc_feature            1..45
                        note = Primer
source                  1..45
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 107
aatgtacagt attgcgtttt ggctacggcg tgcaactcac agaac                  45

SEQ ID NO: 108       moltype = DNA  length = 44
FEATURE              Location/Qualifiers
misc_feature         1..44
                     note = Primer
source               1..44
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 108
aatgtacagt attgcgtttt gaccgacctc ttccagcgct actt                   44

SEQ ID NO: 109       moltype = DNA  length = 44
FEATURE              Location/Qualifiers
misc_feature         1..44
                     note = Primer
source               1..44
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 109
aatgtacagt attgcgtttt gcgggcaggg cttacttacc ttgg                   44

SEQ ID NO: 110       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 110
aatgtacagt attgcgtttt gtagctactg cctgccttcg aagaacgat               49

SEQ ID NO: 111       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 111
aatgtacagt attgcgtttt gtgtgggtgg aaaaagatgt ggttaagaaa caac        54

SEQ ID NO: 112       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 112
aatgtacagt attgcgtttt gcccccatat agcttaatct gatgggcatc              50

SEQ ID NO: 113       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 113
aatgtacagt attgcgtttt ggaaagagca tcaggaacaa gccttgagta c           51

SEQ ID NO: 114       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 114
aatgtacagt attgcgtttt gttgagatgc ctgacaacct ttacaccttt g           51

SEQ ID NO: 115       moltype = DNA  length = 49
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```
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 115
aatgtacagt attgcgtttt gctctagggc tgagggaata tgcatctct              49

SEQ ID NO: 116        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 116
aatgtacagt attgcgtttt gcgtacccag aagacaatgg cctagctat              49

SEQ ID NO: 117        moltype = DNA  length = 45
FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 117
aatgtacagt attgcgtttt ggggcagcac agattccctt aacca                  45

SEQ ID NO: 118        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 118
aatgtacagt attgcgtttt gccatacctt ggctatcccc tgaaagttg              49

SEQ ID NO: 119        moltype = DNA  length = 46
FEATURE               Location/Qualifiers
misc_feature          1..46
                      note = Primer
source                1..46
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 119
aatgtacagt attgcgtttt ggccctgatg ctcatggagt gttcct                 46

SEQ ID NO: 120        moltype = DNA  length = 44
FEATURE               Location/Qualifiers
misc_feature          1..44
                      note = Primer
source                1..44
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 120
aatgtacagt attgcgtttt gcctggtggt tgggagacga ctac                   44

SEQ ID NO: 121        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 121
aatgtacagt attgcgtttt gtgctgacag gacacagaac aagatacct              49

SEQ ID NO: 122        moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 122
aatgtacagt attgcgtttt gggtacaggt atcttgttct gtgtcctgtc ag          52

SEQ ID NO: 123          moltype = DNA  length = 43
FEATURE                 Location/Qualifiers
misc_feature            1..43
                        note = Primer
source                  1..43
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 123
aatgtacagt attgcgtttt ggagtcccgg gctcgattca cag                    43

SEQ ID NO: 124          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 124
aatgtacagt attgcgtttt gctggtcaga gaggtgtgta ctgattgtct             50

SEQ ID NO: 125          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 125
aatgtacagt attgcgtttt gaggaaagat caattacatt cacaagttca cacttct      57

SEQ ID NO: 126          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 126
aatgtacagt attgcgtttt gctgcacagt tcagaggata tttaagctca atgac        55

SEQ ID NO: 127          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 127
aatgtacagt attgcgtttt gcacagaccg tcatgcattt ctgacactc              49

SEQ ID NO: 128          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 128
aatgtacagt attgcgtttt gaggctggta cctgctcttc ttcaatc                47

SEQ ID NO: 129          moltype = DNA  length = 53
FEATURE                 Location/Qualifiers
misc_feature            1..53
                        note = Primer
source                  1..53
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 129
aatgtacagt attgcgtttt gcgaaatcaa acagttgtct atcagagcct gtc          53

SEQ ID NO: 130          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
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                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 130
aatgtacagt attgcgtttt gacaaaagaa aagaagtcat gtctgtatgt ggaaa       55

SEQ ID NO: 131       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 131
aatgtacagt attgcgtttt gtccaggata atacacatca cagtaaataa cactctg     57

SEQ ID NO: 132       moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 132
aatgtacagt attgcgtttt gcatcctctt tgtcatcaag ctacagtctt tttga       55

SEQ ID NO: 133       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 133
aatgtacagt attgcgtttt gctcccattt ttgtgcatct ttgttgctgt c           51

SEQ ID NO: 134       moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 134
aatgtacagt attgcgtttt gcagaactgc ctattcctaa ctgactcatc atttc       55

SEQ ID NO: 135       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 135
aatgtacagt attgcgtttt ggaattctgt ttcatcgctg agtgacactc tttt        54

SEQ ID NO: 136       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 136
aatgtacagt attgcgtttt gtttttacct ttgcttttac ctttttgtac ttgtgac     57

SEQ ID NO: 137       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 137
aatgtacagt attgcgtttt gagaaggagt ctggaataga aaggctaaca gaa         53
```

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SEQ ID NO: 138         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 138
aatgtacagt attgcgtttt gcacaagatg tgccaaggga attgtatgc              49

SEQ ID NO: 139         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 139
aatgtacagt attgcgtttt gaagagtcaa taggtcagag agttttatgt tcttcca      57

SEQ ID NO: 140         moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 140
aatgtacagt attgcgtttt gactgatctt ctcaaagtcg tcatccttca gt           52

SEQ ID NO: 141         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 141
aatgtacagt attgcgtttt gaccctgaga aataatccaa ttacctgtta atcaagg      57

SEQ ID NO: 142         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 142
aatgtacagt attgcgtttt gaaaaggtat tgagtaaaat cagtcttcct tctaccc      57

SEQ ID NO: 143         moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 143
aatgtacagt attgcgtttt gccttcctcc ctctttcttt cataaaacct ctctt        55

SEQ ID NO: 144         moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 144
aatgtacagt attgcgtttt ggccagagcc acccaactct taagg                   45

SEQ ID NO: 145         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 145
aatgtacagt attgcgtttt gtggaagagg aatttaataa cgaacgtttt aagagga     57

SEQ ID NO: 146       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 146
aatgtacagt attgcgtttt ggcatctact gccgaggatg ttccaag               47

SEQ ID NO: 147       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 147
aatgtacagt attgcgtttt gcacagtgag ctcaagtgcg acatca                46

SEQ ID NO: 148       moltype = DNA  length = 43
FEATURE              Location/Qualifiers
misc_feature         1..43
                     note = Primer
source               1..43
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 148
aatgtacagt attgcgtttt gccgactggc catctcctcg tag                   43

SEQ ID NO: 149       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 149
aatgtacagt attgcgtttt ggtaccagcg cgactacgag gagat                 45

SEQ ID NO: 150       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 150
aatgtacagt attgcgtttt gtcttttctg tcaaatggag atgatctctt ctgactc     57

SEQ ID NO: 151       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 151
aatgtacagt attgcgtttt ggggagccca tcatctgcaa aaacatcc               48

SEQ ID NO: 152       moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = Primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 152
aatgtacagt attgcgtttt gaagctgaag aagatgtgga aaagtcccaa tg          52

SEQ ID NO: 153       moltype = DNA  length = 47
```

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FEATURE               Location/Qualifiers
misc_feature          1..47
                      note = Primer
source                1..47
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 153
aatgtacagt attgcgtttt ggcgtgggat gtttttgcag atgatgg                47

SEQ ID NO: 154        moltype = DNA  length = 44
FEATURE               Location/Qualifiers
misc_feature          1..44
                      note = Primer
source                1..44
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 154
aatgtacagt attgcgtttt gcgacgctga ggacgctatg gatg                   44

SEQ ID NO: 155        moltype = DNA  length = 43
FEATURE               Location/Qualifiers
misc_feature          1..43
                      note = Primer
source                1..43
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 155
aatgtacagt attgcgtttt ggctgaggcg cgtcttcgag aag                    43

SEQ ID NO: 156        moltype = DNA  length = 44
FEATURE               Location/Qualifiers
misc_feature          1..44
                      note = Primer
source                1..44
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 156
aatgtacagt attgcgtttt ggcgcttgtc gtgaaagcga acga                   44

SEQ ID NO: 157        moltype = DNA  length = 42
FEATURE               Location/Qualifiers
misc_feature          1..42
                      note = Primer
source                1..42
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 157
aatgtacagt attgcgtttt ggctgcccgc ccagttgtta ct                     42

SEQ ID NO: 158        moltype = DNA  length = 51
FEATURE               Location/Qualifiers
misc_feature          1..51
                      note = Primer
source                1..51
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 158
aatgtacagt attgcgtttt gagactctgg actgatgaag caattctgag t            51

SEQ ID NO: 159        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 159
aatgtacagt attgcgtttt gtcaccggtg acaccttaaa accaaagc               48

SEQ ID NO: 160        moltype = DNA  length = 50
FEATURE               Location/Qualifiers
misc_feature          1..50
                      note = Primer
source                1..50
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 160
aatgtacagt attgcgtttt gggctccttt gtacctcctc catcttgatc            50

SEQ ID NO: 161         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 161
aatgtacagt attgcgtttt ggtcagttgt ctaacaataa caaagatctg ctcttgg     57

SEQ ID NO: 162         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 162
aatgtacagt attgcgtttt gggtgggcag caagaaaaag tccagtaaa              49

SEQ ID NO: 163         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 163
aatgtacagt attgcgtttt ggccaaggct ttctctggca tgatctttt              49

SEQ ID NO: 164         moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 164
aatgtacagt attgcgtttt gggataactt tctcagcatt tccaccagtt tcaag       55

SEQ ID NO: 165         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 165
aatgtacagt attgcgtttt gtgtccctaa gttgagtaaa atgatagaga atgagtc     57

SEQ ID NO: 166         moltype = DNA  length = 50
FEATURE                Location/Qualifiers
misc_feature           1..50
                       note = Primer
source                 1..50
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 166
aatgtacagt attgcgtttt ggctgccaga aatccagcat ccaaaatttg             50

SEQ ID NO: 167         moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 167
aatgtacagt attgcgtttt ggtcgctttc ttttcttagt gccaggaaac t           51

SEQ ID NO: 168         moltype = DNA  length = 50
FEATURE                Location/Qualifiers
misc_feature           1..50
```

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                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 168
aatgtacagt attgcgtttt gacagtcgag acgattcatg agggaacttc            50

SEQ ID NO: 169       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 169
aatgtacagt attgcgtttt gggaaagctc ggcgtgttgg ataagaag              48

SEQ ID NO: 170       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 170
aatgtacagt attgcgtttt gacgccacaa gtgactgaaa gttggaag              48

SEQ ID NO: 171       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 171
aatgtacagt attgcgtttt gtgatgggct ggagatttgg catagttttc            50

SEQ ID NO: 172       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 172
aatgtacagt attgcgtttt gctatgcacc cactttcaac acagttaggt            50

SEQ ID NO: 173       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 173
aatgtacagt attgcgtttt ggcttggtca gaagtgctgt tgttgtc               47

SEQ ID NO: 174       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 174
aatgtacagt attgcgtttt gcgtgggcca gaaagttgtc cacaatg               47

SEQ ID NO: 175       moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = Primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 175
aatgtacagt attgcgtttt ggggatatgg attctcgtgg tagaaggtgt aa          52
```

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```
SEQ ID NO: 176          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 176
aatgtacagt attgcgtttt gctaatcacc aagttccaag tgttcagaat ctcc        54

SEQ ID NO: 177          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 177
aatgtacagt attgcgtttt gaccgtaata accaaggttc atcataggca ttgat       55

SEQ ID NO: 178          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 178
aatgtacagt attgcgtttt gtcccagtgg aagttactat gcaccctat              49

SEQ ID NO: 179          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 179
aatgtacagt attgcgtttt gtgcttatgc ttgtgtttgt gtttcctctt atgg        54

SEQ ID NO: 180          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 180
aatgtacagt attgcgtttt ggcttctgtt tctccttatg cttgttcttc tcac        54

SEQ ID NO: 181          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 181
aatgtacagt attgcgtttt gcctgagtgg tctttttgca ggcaaag                47

SEQ ID NO: 182          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 182
aatgtacagt attgcgtttt gccggccaca aagcttctaa gaacaac                47

SEQ ID NO: 183          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 183
aatgtacagt attgcgtttt ggcggttcat cttgaaggct tggatgt                47

SEQ ID NO: 184       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 184
aatgtacagt attgcgtttt gttcagtgaa atgaaccctt cgaatgacaa g           51

SEQ ID NO: 185       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 185
aatgtacagt attgcgtttt gctcctcctc ctctttgcgt ttcttgtc               48

SEQ ID NO: 186       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 186
aatgtacagt attgcgtttt ggcagcagag aaacaaatga aggacaaaca g           51

SEQ ID NO: 187       moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = Primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 187
aatgtacagt attgcgtttt gtaaggagga ggaagaagac aagaaacgca aa          52

SEQ ID NO: 188       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 188
aatgtacagt attgcgtttt gtaaggcagg tctgtgagca caaaatttgg              50

SEQ ID NO: 189       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 189
aatgtacagt attgcgtttt gtggagctga ccagtgacaa tgacc                   45

SEQ ID NO: 190       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 190
aatgtacagt attgcgtttt gggccaagaa gtcggtggac aagaac                  46

SEQ ID NO: 191       moltype = DNA  length = 43
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```
FEATURE               Location/Qualifiers
misc_feature          1..43
                      note = Primer
source                1..43
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 191
aatgtacagt attgcgtttt ggcgcaggcg gtcattgtca ctg                    43

SEQ ID NO: 192        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 192
aatgtacagt attgcgtttt gttgctgttc ttgtccaccg acttcttg               48

SEQ ID NO: 193        moltype = DNA  length = 42
FEATURE               Location/Qualifiers
misc_feature          1..42
                      note = Primer
source                1..42
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 193
aatgtacagt attgcgtttt ggcagtgcgc gatctggaac tg                     42

SEQ ID NO: 194        moltype = DNA  length = 42
FEATURE               Location/Qualifiers
misc_feature          1..42
                      note = Primer
source                1..42
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 194
aatgtacagt attgcgtttt gcggcggcga ctttgactac cc                     42

SEQ ID NO: 195        moltype = DNA  length = 45
FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 195
aatgtacagt attgcgtttt ggagcacgag acgtccatcg acatc                  45

SEQ ID NO: 196        moltype = DNA  length = 43
FEATURE               Location/Qualifiers
misc_feature          1..43
                      note = Primer
source                1..43
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 196
aatgtacagt attgcgtttt gcggccagga actcgtcgtt gaa                    43

SEQ ID NO: 197        moltype = DNA  length = 45
FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 197
aatgtacagt attgcgtttt ggccatgccg ggagaactct aactc                  45

SEQ ID NO: 198        moltype = DNA  length = 54
FEATURE               Location/Qualifiers
misc_feature          1..54
                      note = Primer
source                1..54
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 198
aatgtacagt attgcgtttt gtgtaaccct cctaagtgtt catacgttgt cttg        54

SEQ ID NO: 199         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 199
aatgtacagt attgcgtttt ggtcttggtc tctgttatat cttgagtcta gaacagt     57

SEQ ID NO: 200         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 200
aatgtacagt attgcgtttt gcaggagaac atggaggcga gaagaaaat              49

SEQ ID NO: 201         moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 201
aatgtacagt attgcgtttt ggggaaagat tggatgccgg gaatcaac               48

SEQ ID NO: 202         moltype = DNA  length = 46
FEATURE                Location/Qualifiers
misc_feature           1..46
                       note = Primer
source                 1..46
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 202
aatgtacagt attgcgtttt gcggaggctt gattaggtag gaggtg                 46

SEQ ID NO: 203         moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 203
aatgtacagt attgcgtttt ggcggcagct caacgagaat aaaca                  45

SEQ ID NO: 204         moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 204
aatgtacagt attgcgtttt ggcccgcatc cttactccgc ttatc                  45

SEQ ID NO: 205         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 205
aatgtacagt attgcgtttt ggctggtttc aaggtaagtg gactcttcc              49

SEQ ID NO: 206         moltype = DNA  length = 47
FEATURE                Location/Qualifiers
misc_feature           1..47
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                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 206
aatgtacagt attgcgtttt ggggaatgac tgacggagaa tcccaac                47

SEQ ID NO: 207       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 207
aatgtacagt attgcgtttt gctaagaccg agagcctgta ggagcttt               48

SEQ ID NO: 208       moltype = DNA  length = 42
FEATURE              Location/Qualifiers
misc_feature         1..42
                     note = Primer
source               1..42
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 208
aatgtacagt attgcgtttt ggccgggctt gtctggtcat ct                     42

SEQ ID NO: 209       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 209
aatgtacagt attgcgtttt gcagctcacc tccaaaaagg caaaattctt g            51

SEQ ID NO: 210       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 210
aatgtacagt attgcgtttt ggcaggaggc catgatggat ttcttcaa               48

SEQ ID NO: 211       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 211
aatgtacagt attgcgtttt gcatgagtga aaggaaagag gaaatcccaa tcc          53

SEQ ID NO: 212       moltype = DNA  length = 56
FEATURE              Location/Qualifiers
misc_feature         1..56
                     note = Primer
source               1..56
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 212
aatgtacagt attgcgtttt gcctatcttc cacagtactt acacaacttc ctaagc       56

SEQ ID NO: 213       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 213
aatgtacagt attgcgtttt gctcgccgta gactgtccag gtttt                  45
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SEQ ID NO: 214          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 214
aatgtacagt attgcgtttt gctcacctga tccgtgacgt tgatgtc                47

SEQ ID NO: 215          moltype = DNA  length = 44
FEATURE                 Location/Qualifiers
misc_feature            1..44
                        note = Primer
source                  1..44
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 215
aatgtacagt attgcgtttt ggccctgatg gactctcggc tact                   44

SEQ ID NO: 216          moltype = DNA  length = 53
FEATURE                 Location/Qualifiers
misc_feature            1..53
                        note = Primer
source                  1..53
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 216
aatgtacagt attgcgtttt ggagaaagat caggaacact tgtcccctac tag          53

SEQ ID NO: 217          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 217
aatgtacagt attgcgtttt ggtcctccac gatctcctca tactcctc               48

SEQ ID NO: 218          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 218
aatgtacagt attgcgtttt gtcgatggac ttgacaagcc cgtactt                47

SEQ ID NO: 219          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 219
aatgtacagt attgcgtttt gctggacgac gaggagtatg aggagatc               48

SEQ ID NO: 220          moltype = DNA  length = 46
FEATURE                 Location/Qualifiers
misc_feature            1..46
                        note = Primer
source                  1..46
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 220
aatgtacagt attgcgtttt gtaccagaag tcccggcggt gataag                 46

SEQ ID NO: 221          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 221
aatgtacagt attgcgtttt ggttcacctc tgtgtttgac tgccagaaa              49

SEQ ID NO: 222       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 222
aatgtacagt attgcgtttt gcaatgagta ttctcttcat ttcaggtcag ttgattt      57

SEQ ID NO: 223       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 223
aatgtacagt attgcgtttt gggctgcttt cttgaaggct attgggtat              49

SEQ ID NO: 224       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 224
aatgtacagt attgcgtttt gaggagactg gaattctcga ataaggatta aca          53

SEQ ID NO: 225       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 225
aatgtacagt attgcgtttt ggcatagtta aaacctgtgt ttggttttgt aggtctt      57

SEQ ID NO: 226       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 226
aatgtacagt attgcgtttt gctctgtgtt ggcggatacc cttccata               48

SEQ ID NO: 227       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 227
aatgtacagt attgcgtttt gggcattcct tctttattgc ccttcttaaa agc          53

SEQ ID NO: 228       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 228
aatgtacagt attgcgtttt ggctgctggt ctggctacta tgatctctac             50

SEQ ID NO: 229       moltype = DNA  length = 53
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FEATURE               Location/Qualifiers
misc_feature          1..53
                      note = Primer
source                1..53
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 229
aatgtacagt attgcgtttt ggcacacagc ttttaagaag ggcaataaag aag         53

SEQ ID NO: 230        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 230
aatgtacagt attgcgtttt gtgtatgttt aattctgtac atgagcattt catcagt      57

SEQ ID NO: 231        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 231
aatgtacagt attgcgtttt gatttcatac cttgcttaat gggtgtagat accaaaa      57

SEQ ID NO: 232        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 232
aatgtacagt attgcgtttt gttggcgtca aatgtgccac tatcactc                48

SEQ ID NO: 233        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 233
aatgtacagt attgcgtttt gttctctttc aagctatgat ttaggcatag agaatcg      57

SEQ ID NO: 234        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 234
aatgtacagt attgcgtttt gctgcagttg taggttataa ctatccattt gtctgaa      57

SEQ ID NO: 235        moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 235
aatgtacagt attgcgtttt gccctaggtc agatcaccca gtcagttaaa ac          52

SEQ ID NO: 236        moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 236
aatgtacagt attgcgtttt gtggttaaag gtcagcccac ttaccagata tg          52

SEQ ID NO: 237          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 237
aatgtacagt attgcgtttt ggggtatgct ccccatttag aggataagg               49

SEQ ID NO: 238          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 238
aatgtacagt attgcgtttt gacgtcagat ctacagcgaa cacaactact              50

SEQ ID NO: 239          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 239
aatgtacagt attgcgtttt gagtggtgcc agactcacat tcagttctaa              50

SEQ ID NO: 240          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 240
aatgtacagt attgcgtttt gcttggccag ttcctttctc taatgtatca tctc          54

SEQ ID NO: 241          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 241
aatgtacagt attgcgtttt gaagttttct tgtctagtat cactttccct catagg        56

SEQ ID NO: 242          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 242
aatgtacagt attgcgtttt ggggctcaac agatggtatg tgttctctg               49

SEQ ID NO: 243          moltype = DNA  length = 54
FEATURE                 Location/Qualifiers
misc_feature            1..54
                        note = Primer
source                  1..54
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 243
aatgtacagt attgcgtttt ggctctcgtt tctaacagtt ctttgcattg gata          54

SEQ ID NO: 244          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
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                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 244
aatgtacagt attgcgtttt ggaggtgacc ttcaaagtca gaggctgtat            50

SEQ ID NO: 245       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 245
aatgtacagt attgcgtttt ggagcaacca tcccatctgt ccttgtaac              49

SEQ ID NO: 246       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 246
aatgtacagt attgcgtttt gggacaagga tgagaaaccc aattggaacc             50

SEQ ID NO: 247       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 247
aatgtacagt attgcgtttt gcggtccgcc aaaagatccc agattc                 46

SEQ ID NO: 248       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 248
aatgtacagt attgcgtttt gggaggccac taacccactt gtgatg                 46

SEQ ID NO: 249       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 249
aatgtacagt attgcgtttt gtccagtttc ctagaggatg taatgggatt tgtc         54

SEQ ID NO: 250       moltype = DNA  length = 52
FEATURE              Location/Qualifiers
misc_feature         1..52
                     note = Primer
source               1..52
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 250
aatgtacagt attgcgtttt gtcacatttg gagatgagaa acgaggtgtt ct           52

SEQ ID NO: 251       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 251
aatgtacagt attgcgtttt gcccttggcc tgtaacattg ctctgatc               48
```

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SEQ ID NO: 252          moltype = DNA  length = 52
FEATURE                 Location/Qualifiers
misc_feature            1..52
                        note = Primer
source                  1..52
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 252
aatgtacagt attgcgtttt gcacctcgtt tctcatctcc aaatgtgatc tc          52

SEQ ID NO: 253          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 253
aatgtacagt attgcgtttt gccagtagct ttcctgttct cggcatt                47

SEQ ID NO: 254          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 254
aatgtacagt attgcgtttt ggcagcgtca agaatgagaa gacttttgtg              50

SEQ ID NO: 255          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 255
aatgtacagt attgcgtttt gttgcccttc tggaaattac cccgaga                 47

SEQ ID NO: 256          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 256
aatgtacagt attgcgtttt gagttccacc agctttaatt attcctctag ctctc        55

SEQ ID NO: 257          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 257
aatgtacagt attgcgtttt ggtttcccat ggccataatt tattatctca ccacaa       56

SEQ ID NO: 258          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 258
aatgtacagt attgcgtttt ggtcacgatg actgtattgg accctcaa                48

SEQ ID NO: 259          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 259
aatgtacagt attgcgtttt gtccagacct ttgctttaga ttggcaatta ttactg      56

SEQ ID NO: 260       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 260
aatgtacagt attgcgtttt gccctaacaa cacagaagca aagcgttctt t           51

SEQ ID NO: 261       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 261
aatgtacagt attgcgtttt gcgccctcct accacctgta ctacg                  45

SEQ ID NO: 262       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 262
aatgtacagt attgcgtttt gactatccag gcgccttcac ctactc                 46

SEQ ID NO: 263       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 263
aatgtacagt attgcgtttt gctcctaggc ggtatcatcc tgggtag                47

SEQ ID NO: 264       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 264
aatgtacagt attgcgtttt gtctgattct cttcagatac aaggcagatc c           51

SEQ ID NO: 265       moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 265
aatgtacagt attgcgtttt ggcagatact tggacttgag taggcttatt aaacc       55

SEQ ID NO: 266       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 266
aatgtacagt attgcgtttt ggcggctcta taaagaattg tccttatttt cgaactt     57

SEQ ID NO: 267       moltype = DNA  length = 48
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FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 267
aatgtacagt attgcgtttt ggttcgaggc ctttctctga gcatcaag              48

SEQ ID NO: 268        moltype = DNA  length = 51
FEATURE               Location/Qualifiers
misc_feature          1..51
                      note = Primer
source                1..51
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 268
aatgtacagt attgcgtttt gacatcggca gaaactagat gatcagacca a          51

SEQ ID NO: 269        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 269
aatgtacagt attgcgtttt gtttaggaaa tccacaatac ttttttctgat ctcttcc    57

SEQ ID NO: 270        moltype = DNA  length = 53
FEATURE               Location/Qualifiers
misc_feature          1..53
                      note = Primer
source                1..53
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 270
aatgtacagt attgcgtttt ggccaccaac ctcattctgt tttgttctct atc        53

SEQ ID NO: 271        moltype = DNA  length = 51
FEATURE               Location/Qualifiers
misc_feature          1..51
                      note = Primer
source                1..51
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 271
aatgtacagt attgcgtttt gctgcatttg tcctttgact ggtgtttagg t          51

SEQ ID NO: 272        moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 272
aatgtacagt attgcgtttt gcttcgaccg acaaacctga ggtcattaaa tc         52

SEQ ID NO: 273        moltype = DNA  length = 46
FEATURE               Location/Qualifiers
misc_feature          1..46
                      note = Primer
source                1..46
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 273
aatgtacagt attgcgtttt gccccacatc ccaagctagg aagacc                 46

SEQ ID NO: 274        moltype = DNA  length = 45
FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 274
aatgtacagt attgcgtttt gcgggccagt accttgaaag cgatg                45

SEQ ID NO: 275         moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 275
aatgtacagt attgcgtttt gctaactcaa tcggcttgtt gtgatgcgta t          51

SEQ ID NO: 276         moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 276
aatgtacagt attgcgtttt gccctcctgg actgttagta acttagtctc c          51

SEQ ID NO: 277         moltype = DNA  length = 42
FEATURE                Location/Qualifiers
misc_feature           1..42
                       note = Primer
source                 1..42
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 277
aatgtacagt attgcgtttt gccctccgag ctccgcgaaa at                   42

SEQ ID NO: 278         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 278
aatgtacagt attgcgtttt ggtgctaaaa agtgtaagaa gaaatgagct agcaaaa    57

SEQ ID NO: 279         moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 279
aatgtacagt attgcgtttt gcatatgcct cagtttgaat tcctctcaca aacaa      55

SEQ ID NO: 280         moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 280
aatgtacagt attgcgtttt ggggagaaga aagagagatg tagggctaga g          51

SEQ ID NO: 281         moltype = DNA  length = 54
FEATURE                Location/Qualifiers
misc_feature           1..54
                       note = Primer
source                 1..54
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 281
aatgtacagt attgcgtttt ggcaagcact tctgtttttg tcttttcagt ttcg       54

SEQ ID NO: 282         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
```

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                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 282
aatgtacagt attgcgtttt gtctctgata tacttggatt ggtaattgag aaagtct     57

SEQ ID NO: 283       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 283
aatgtacagt attgcgtttt ggtttgatat cttcccagca aaataatcag ctctcat     57

SEQ ID NO: 284       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 284
aatgtacagt attgcgtttt gtagccaacc tcttttcgat gagctcacta g           51

SEQ ID NO: 285       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 285
aatgtacagt attgcgtttt gtggaacaga caaactatcg actgaagttg t           51

SEQ ID NO: 286       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 286
aatgtacagt attgcgtttt ggaggctgag tgcaaatttg gtctggaa               48

SEQ ID NO: 287       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 287
aatgtacagt attgcgtttt ggatggtggt ggttgtctct gatgattacc             50

SEQ ID NO: 288       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 288
aatgtacagt attgcgtttt ggcaaggcga gtccagaacc aagatt                 46

SEQ ID NO: 289       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 289
aatgtacagt attgcgtttt gtcagaagcg actgatcccc atcaagt                47
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SEQ ID NO: 290          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 290
aatgtacagt attgcgtttt gcatatggtc acatcacctt aactaaaccc atgttt      56

SEQ ID NO: 291          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 291
aatgtacagt attgcgtttt gtttctcggt actgtttatt ttgaacaaaa ccaatcc     57

SEQ ID NO: 292          moltype = DNA  length = 51
FEATURE                 Location/Qualifiers
misc_feature            1..51
                        note = Primer
source                  1..51
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 292
aatgtacagt attgcgtttt gcctcctccc caaattccag gaacaatatg a           51

SEQ ID NO: 293          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 293
aatgtacagt attgcgtttt gtgtgcgtca ttttatttgg gaaaatttga tactaac     57

SEQ ID NO: 294          moltype = DNA  length = 46
FEATURE                 Location/Qualifiers
misc_feature            1..46
                        note = Primer
source                  1..46
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 294
aatgtacagt attgcgtttt gcatgcagga gaagtcatcc cccttc                 46

SEQ ID NO: 295          moltype = DNA  length = 51
FEATURE                 Location/Qualifiers
misc_feature            1..51
                        note = Primer
source                  1..51
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 295
aatgtacagt attgcgtttt gtctgaaaac tggtggttgc ctctaggtta a           51

SEQ ID NO: 296          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 296
aatgtacagt attgcgtttt ggccccttcc ttgctcttct tggacttg               48

SEQ ID NO: 297          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 297
aatgtacagt attgcgtttt gccaagccaa gccaagctgg atattgtg              48

SEQ ID NO: 298       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 298
aatgtacagt attgcgtttt gcactcacat tgtgcagctt gtagtagag              49

SEQ ID NO: 299       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 299
aatgtacagt attgcgtttt ggcaaagcgt ctgcatttga aggagttt              48

SEQ ID NO: 300       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 300
aatgtacagt attgcgtttt gccctcccga gaacttgccg gttaa                  45

SEQ ID NO: 301       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 301
aatgtacagt attgcgtttt ggctccccac cacaaaaacg caaatg                 46

SEQ ID NO: 302       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 302
aatgtacagt attgcgtttt ggtgtcactg acggagagca tgaagatg               48

SEQ ID NO: 303       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 303
aatgtacagt attgcgtttt gccacccaaa gaagtgtctc ctgacc                 46

SEQ ID NO: 304       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 304
aatgtacagt attgcgtttt gtccgtcagt gacacctggt acttgac                47

SEQ ID NO: 305       moltype = DNA  length = 48
```

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FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 305
aatgtacagt attgcgtttt gccctagctc tgcctaccct gatctttc              48

SEQ ID NO: 306        moltype = DNA  length = 47
FEATURE               Location/Qualifiers
misc_feature          1..47
                      note = Primer
source                1..47
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 306
aatgtacagt attgcgtttt gacgaggtgg acgtcttctt caatcac               47

SEQ ID NO: 307        moltype = DNA  length = 43
FEATURE               Location/Qualifiers
misc_feature          1..43
                      note = Primer
source                1..43
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 307
aatgtacagt attgcgtttt ggccctgcga gtcgaggtga ttg                   43

SEQ ID NO: 308        moltype = DNA  length = 53
FEATURE               Location/Qualifiers
misc_feature          1..53
                      note = Primer
source                1..53
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 308
aatgtacagt attgcgtttt gccatgactc tcaggaattg gccctatact tag         53

SEQ ID NO: 309        moltype = DNA  length = 56
FEATURE               Location/Qualifiers
misc_feature          1..56
                      note = Primer
source                1..56
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 309
aatgtacagt attgcgtttt gcttgggacc ttcatttcta tataacccct atctgg      56

SEQ ID NO: 310        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 310
aatgtacagt attgcgtttt gtgccaggaa acttttcatt gtgcctctc              49

SEQ ID NO: 311        moltype = DNA  length = 50
FEATURE               Location/Qualifiers
misc_feature          1..50
                      note = Primer
source                1..50
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 311
aatgtacagt attgcgtttt ggttacccca tggaacttac caagcactag             50

SEQ ID NO: 312        moltype = DNA  length = 54
FEATURE               Location/Qualifiers
misc_feature          1..54
                      note = Primer
source                1..54
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 312
aatgtacagt attgcgtttt ggtatgaaat tcgctggagg gtcattgaat caat        54

SEQ ID NO: 313         moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 313
aatgtacagt attgcgtttt gcaggaagga gcacttacgt tttagcatct tc          52

SEQ ID NO: 314         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 314
aatgtacagt attgcgtttt ggattttgag aaattccctt aatatcccca tgctcaa     57

SEQ ID NO: 315         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 315
aatgtacagt attgcgtttt gcacaaccac atgtgtccag tgaaaatcc              49

SEQ ID NO: 316         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 316
aatgtacagt attgcgtttt gtgctttcat cagcagggtt caatccaaa              49

SEQ ID NO: 317         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 317
aatgtacagt attgcgtttt gcatttacat catcacagag tattgcttct atggaga     57

SEQ ID NO: 318         moltype = DNA  length = 56
FEATURE                Location/Qualifiers
misc_feature           1..56
                       note = Primer
source                 1..56
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 318
aatgtacagt attgcgtttt ggtgatctct ggatgtcgga atatttagaa acctct      56

SEQ ID NO: 319         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 319
aatgtacagt attgcgtttt gatcttttga aaacaatggt gactacatgg acatgaa     57

SEQ ID NO: 320         moltype = DNA  length = 54
FEATURE                Location/Qualifiers
misc_feature           1..54
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                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 320
aatgtacagt attgcgtttt gggtctaaaa aggtctgtgt tccttgaact taca        54

SEQ ID NO: 321       moltype = DNA  length = 56
FEATURE              Location/Qualifiers
misc_feature         1..56
                     note = Primer
source               1..56
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 321
aatgtacagt attgcgtttt gccagcacca atacatttaa tttcttttct gcagac      56

SEQ ID NO: 322       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 322
aatgtacagt attgcgtttt ggctacagat ggcttgatcc tgagtcattt c           51

SEQ ID NO: 323       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 323
aatgtacagt attgcgtttt ggtcaggccc ataccaaggg aaaagatc               48

SEQ ID NO: 324       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 324
aatgtacagt attgcgtttt gacactgagt gatgtctggt cttatggcat t           51

SEQ ID NO: 325       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 325
aatgtacagt attgcgtttt gcactgagcg tttgttagtc ctggtgtttt             50

SEQ ID NO: 326       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 326
aatgtacagt attgcgtttt gcagattctc cacaatctca ctcaggtggt aaa         53

SEQ ID NO: 327       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 327
aatgtacagt attgcgtttt gccccacagc tacgagatca tggtgaaat              49
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SEQ ID NO: 328          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 328
aatgtacagt attgcgtttt gtctctattc atttttgagg tttggttgtt aacactt     57

SEQ ID NO: 329          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 329
aatgtacagt attgcgtttt ggggagtgca ccattatcgg gaaaatgg               48

SEQ ID NO: 330          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 330
aatgtacagt attgcgtttt ggcttattct cattcgtttc atccaggatc tcaaaa      56

SEQ ID NO: 331          moltype = DNA  length = 46
FEATURE                 Location/Qualifiers
misc_feature            1..46
                        note = Primer
source                  1..46
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 331
aatgtacagt attgcgtttt ggggcgacga gattaggctg ttatgc                 46

SEQ ID NO: 332          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 332
aatgtacagt attgcgtttt gcccctctgc attataagca gtgccaaaa               49

SEQ ID NO: 333          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 333
aatgtacagt attgcgtttt ggcccacatc gttgtaagcc ttacattcaa             50

SEQ ID NO: 334          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 334
aatgtacagt attgcgtttt gccgtttgga aagctagtgg ttcagagttc             50

SEQ ID NO: 335          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 335
aatgtacagt attgcgtttt ggagatccca tcctgccaaa gtttgtgatt            50

SEQ ID NO: 336       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 336
aatgtacagt attgcgtttt gggaaagccc ctgtttcata ctgaccaaaa            50

SEQ ID NO: 337       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 337
aatgtacagt attgcgtttt gctttctccc cacagaaacc catgtatgaa g           51

SEQ ID NO: 338       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 338
aatgtacagt attgcgtttt ggtttgccag ttgtgctttt tgctaaaatg c           51

SEQ ID NO: 339       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 339
aatgtacagt attgcgtttt gccctcccac cctcaggact ataccaat              48

SEQ ID NO: 340       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 340
aatgtacagt attgcgtttt gtgctcggca gattggtata gtcctg                46

SEQ ID NO: 341       moltype = DNA  length = 50
FEATURE              Location/Qualifiers
misc_feature         1..50
                     note = Primer
source               1..50
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 341
aatgtacagt attgcgtttt gggcatcctc tgtcctatct cccagataca             50

SEQ ID NO: 342       moltype = DNA  length = 56
FEATURE              Location/Qualifiers
misc_feature         1..56
                     note = Primer
source               1..56
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 342
aatgtacagt attgcgtttt gaggttttat actaaactta ctttgactgg gtttgg      56

SEQ ID NO: 343       moltype = DNA  length = 45
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FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 343
aatgtacagt attgcgtttt gcccccagag gtaagcgtca tatgg                  45

SEQ ID NO: 344        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 344
aatgtacagt attgcgtttt ggcacaggga agtaggtact gggagattg              49

SEQ ID NO: 345        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 345
aatgtacagt attgcgtttt gaggcctgca aggttttaac tggaccta               48

SEQ ID NO: 346        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 346
aatgtacagt attgcgtttt gcgggagctg ataagtggta cctgtatgt              49

SEQ ID NO: 347        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 347
aatgtacagt attgcgtttt ggaaaagggt cccaggtagg tccagttaa              49

SEQ ID NO: 348        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 348
aatgtacagt attgcgtttt gctctcggtg tatttctcta cttacctgta ataatgc     57

SEQ ID NO: 349        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 349
aatgtacagt attgcgtttt gtttattgat gtctatgaag tgttgtggtt ccttaac     57

SEQ ID NO: 350        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 350
aatgtacagt attgcgtttt gcagaaaaca agctgccgca aagttctac              49

SEQ ID NO: 351         moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 351
aatgtacagt attgcgtttt gcaggtgttg cgatgatgtc actgtacg               48

SEQ ID NO: 352         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 352
aatgtacagt attgcgtttt gtcatttttc attggacttg ttttgtcagc tttttgg      57

SEQ ID NO: 353         moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 353
aatgtacagt attgcgtttt ggttagcccc aatatgaaaa ataaagctgg ttgga        55

SEQ ID NO: 354         moltype = DNA  length = 53
FEATURE                Location/Qualifiers
misc_feature           1..53
                       note = Primer
source                 1..53
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 354
aatgtacagt attgcgtttt gctggttgga ggtttttgct aaatctggaa tga          53

SEQ ID NO: 355         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 355
aatgtacagt attgcgtttt gttctttttg actagaaaac ttcagccact gtgtatt      57

SEQ ID NO: 356         moltype = DNA  length = 55
FEATURE                Location/Qualifiers
misc_feature           1..55
                       note = Primer
source                 1..55
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 356
aatgtacagt attgcgtttt gcatatgacc aattgcagat gagcccatta ttgaa        55

SEQ ID NO: 357         moltype = DNA  length = 51
FEATURE                Location/Qualifiers
misc_feature           1..51
                       note = Primer
source                 1..51
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 357
aatgtacagt attgcgtttt gaggcatagc tgactcatct atgtttgttc t            51

SEQ ID NO: 358         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
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                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 358
aatgtacagt attgcgtttt gttcctcatt tctttcactc tgacagtata aaggtaa     57

SEQ ID NO: 359       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 359
aatgtacagt attgcgtttt ggaactattc caacagaaca aaccgataac atca        54

SEQ ID NO: 360       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 360
aatgtacagt attgcgtttt gtggatagca agacaattag agcccaactt agt         53

SEQ ID NO: 361       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 361
aatgtacagt attgcgtttt gctactcctc ctgtctcttt ccacatcatc aatt        54

SEQ ID NO: 362       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 362
aatgtacagt attgcgtttt gaggacctta tgttgtatgc tgtataaatc taaaggt     57

SEQ ID NO: 363       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 363
aatgtacagt attgcgtttt ggtttgtcat cttctatggt aagtatcttt ctggatg     57

SEQ ID NO: 364       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 364
aatgtacagt attgcgtttt gtggaggaga aacagataaa agttgagtat acgttta     57

SEQ ID NO: 365       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 365
aatgtacagt attgcgtttt ggaggatgac gacatgttag taagcactac tact        54
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SEQ ID NO: 366          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 366
aatgtacagt attgcgtttt gattccacca tcatttcctt ctccaaaatt atcatcc     57

SEQ ID NO: 367          moltype = DNA  length = 53
FEATURE                 Location/Qualifiers
misc_feature            1..53
                        note = Primer
source                  1..53
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 367
aatgtacagt attgcgtttt gctcaaaagc actgccttct ctcattatct cac         53

SEQ ID NO: 368          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 368
aatgtacagt attgcgtttt gaatgtattt gaccttcttt taaagtgaca tcgatgt     57

SEQ ID NO: 369          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 369
aatgtacagt attgcgtttt gtgatgttcc caacttcttc tctcatggtt atctc       55

SEQ ID NO: 370          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 370
aatgtacagt attgcgtttt gccctctgat ccctagataa tttatgggta gctaga      56

SEQ ID NO: 371          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 371
aatgtacagt attgcgtttt gcacgaaatg caggttttgg aatatgatta atgtt       55

SEQ ID NO: 372          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 372
aatgtacagt attgcgtttt ggaacaatgt tctacgcaca ttttgttctc agtaaa      56

SEQ ID NO: 373          moltype = DNA  length = 50
FEATURE                 Location/Qualifiers
misc_feature            1..50
                        note = Primer
source                  1..50
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 373
aatgtacagt attgcgtttt gtccacgctg ctctctaaat tacactcgaa            50

SEQ ID NO: 374       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 374
aatgtacagt attgcgtttt gacgtagaac acatttcatt ttactcctct ttgg        54

SEQ ID NO: 375       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 375
aatgtacagt attgcgtttt ggtcacatga atgtaaatca agaaaacaga tgttgtt     57

SEQ ID NO: 376       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 376
aatgtacagt attgcgtttt gttctgaact atttatggac aacagtcaaa caacaat     57

SEQ ID NO: 377       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 377
aatgtacagt attgcgtttt gtgaagccat tgcgagaact ttatccataa gtatttc     57

SEQ ID NO: 378       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 378
aatgtacagt attgcgtttt ggccagagca catgaataaa tgagcatcca t           51

SEQ ID NO: 379       moltype = DNA  length = 53
FEATURE              Location/Qualifiers
misc_feature         1..53
                     note = Primer
source               1..53
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 379
aatgtacagt attgcgtttt gggaagctct cagggtacaa attctcagat cat         53

SEQ ID NO: 380       moltype = DNA  length = 54
FEATURE              Location/Qualifiers
misc_feature         1..54
                     note = Primer
source               1..54
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 380
aatgtacagt attgcgtttt gctcagggta caaattctca gatcatcagt cctc        54

SEQ ID NO: 381       moltype = DNA  length = 49
```

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```
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 381
aatgtacagt attgcgtttt gctctacaca agcttccttt ccgtcatgc              49

SEQ ID NO: 382        moltype = DNA  length = 52
FEATURE               Location/Qualifiers
misc_feature          1..52
                      note = Primer
source                1..52
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 382
aatgtacagt attgcgtttt gcccttcaga tcttctcagc attcgagaga tc          52

SEQ ID NO: 383        moltype = DNA  length = 49
FEATURE               Location/Qualifiers
misc_feature          1..49
                      note = Primer
source                1..49
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 383
aatgtacagt attgcgtttt gaatcgaagc gctacctgat tccaattcc              49

SEQ ID NO: 384        moltype = DNA  length = 46
FEATURE               Location/Qualifiers
misc_feature          1..46
                      note = Primer
source                1..46
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 384
aatgtacagt attgcgtttt gccgaccgta actattcggt gcgttg                 46

SEQ ID NO: 385        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 385
aatgtacagt attgcgtttt gacattctat ccaagctgtg ttctatcttg agaaact      57

SEQ ID NO: 386        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 386
aatgtacagt attgcgtttt gcgagtgagg gttttcgtgg ttcacatc               48

SEQ ID NO: 387        moltype = DNA  length = 45
FEATURE               Location/Qualifiers
misc_feature          1..45
                      note = Primer
source                1..45
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 387
aatgtacagt attgcgtttt gcgtgggtcc cagtctgcag ttaag                  45

SEQ ID NO: 388        moltype = DNA  length = 44
FEATURE               Location/Qualifiers
misc_feature          1..44
                      note = Primer
source                1..44
                      mol_type = other DNA
                      organism = synthetic construct
```

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SEQUENCE: 388
aatgtacagt attgcgtttt ggctcagagc cgttccgaga tctt                  44

SEQ ID NO: 389         moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 389
aatgtacagt attgcgtttt ggcgttccat ctcccacttg tcgtagtt               48

SEQ ID NO: 390         moltype = DNA  length = 49
FEATURE                Location/Qualifiers
misc_feature           1..49
                       note = Primer
source                 1..49
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 390
aatgtacagt attgcgtttt gctggccgag ttggttcatc atcattcaa               49

SEQ ID NO: 391         moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 391
aatgtacagt attgcgtttt gtatggtgtg tcccccaact acgacaag               48

SEQ ID NO: 392         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 392
aatgtacagt attgcgtttt gtgaaaagca cttcctgaaa taatttcacc ttcgttt      57

SEQ ID NO: 393         moltype = DNA  length = 47
FEATURE                Location/Qualifiers
misc_feature           1..47
                       note = Primer
source                 1..47
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 393
aatgtacagt attgcgtttt gaggtactcc atggctgacg agatctg                47

SEQ ID NO: 394         moltype = DNA  length = 48
FEATURE                Location/Qualifiers
misc_feature           1..48
                       note = Primer
source                 1..48
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 394
aatgtacagt attgcgtttt gttgcctttg ttccaaggtc caatgtgt               48

SEQ ID NO: 395         moltype = DNA  length = 43
FEATURE                Location/Qualifiers
misc_feature           1..43
                       note = Primer
source                 1..43
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 395
aatgtacagt attgcgtttt gcgtccccgc attccaacgt ctc                    43

SEQ ID NO: 396         moltype = DNA  length = 42
FEATURE                Location/Qualifiers
misc_feature           1..42
```

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                     note = Primer
source               1..42
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 396
aatgtacagt attgcgtttt gggcgcgccg tttacttgaa gg                    42

SEQ ID NO: 397       moltype = DNA  length = 44
FEATURE              Location/Qualifiers
misc_feature         1..44
                     note = Primer
source               1..44
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 397
aatgtacagt attgcgtttt ggcctggcgg tgcacactat tctg                  44

SEQ ID NO: 398       moltype = DNA  length = 48
FEATURE              Location/Qualifiers
misc_feature         1..48
                     note = Primer
source               1..48
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 398
aatgtacagt attgcgtttt gaggtgcagc cacaaaactt acagatgc              48

SEQ ID NO: 399       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 399
aatgtacagt attgcgtttt ggtgccgaac caatacaacc ctctg                 45

SEQ ID NO: 400       moltype = DNA  length = 43
FEATURE              Location/Qualifiers
misc_feature         1..43
                     note = Primer
source               1..43
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 400
aatgtacagt attgcgtttt ggggcgggtc caccagtttg aat                   43

SEQ ID NO: 401       moltype = DNA  length = 44
FEATURE              Location/Qualifiers
misc_feature         1..44
                     note = Primer
source               1..44
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 401
aatgtacagt attgcgtttt gccgcagagg gttgtattgg ttcg                  44

SEQ ID NO: 402       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 402
aatgtacagt attgcgtttt gagccactcg cattgaccat tcaaact                47

SEQ ID NO: 403       moltype = DNA  length = 44
FEATURE              Location/Qualifiers
misc_feature         1..44
                     note = Primer
source               1..44
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 403
aatgtacagt attgcgtttt gccacgtctg acaggtagcc atgg                  44
```

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SEQ ID NO: 404          moltype = DNA  length = 45
FEATURE                 Location/Qualifiers
misc_feature            1..45
                        note = Primer
source                  1..45
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 404
aatgtacagt attgcgtttt ggtgaggctg ctggacgagt acaac                  45

SEQ ID NO: 405          moltype = DNA  length = 43
FEATURE                 Location/Qualifiers
misc_feature            1..43
                        note = Primer
source                  1..43
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 405
aatgtacagt attgcgtttt gcgcaccagg ttgtactcgt cca                    43

SEQ ID NO: 406          moltype = DNA  length = 46
FEATURE                 Location/Qualifiers
misc_feature            1..46
                        note = Primer
source                  1..46
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 406
aatgtacagt attgcgtttt gccgcctttg tgcttctgtt cttcgt                 46

SEQ ID NO: 407          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 407
aatgtacagt attgcgtttt gctgattaat cgcgtagaaa atgaccttat tttggag      57

SEQ ID NO: 408          moltype = DNA  length = 49
FEATURE                 Location/Qualifiers
misc_feature            1..49
                        note = Primer
source                  1..49
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 408
aatgtacagt attgcgtttt ggctccatcg tctacctgga gattgacaa               49

SEQ ID NO: 409          moltype = DNA  length = 45
FEATURE                 Location/Qualifiers
misc_feature            1..45
                        note = Primer
source                  1..45
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 409
aatgtacagt attgcgtttt gtctgcacgg cctcgatctt gtagg                  45

SEQ ID NO: 410          moltype = DNA  length = 48
FEATURE                 Location/Qualifiers
misc_feature            1..48
                        note = Primer
source                  1..48
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 410
aatgtacagt attgcgtttt ggccagcaga tgatcttccc ctactacg               48

SEQ ID NO: 411          moltype = DNA  length = 44
FEATURE                 Location/Qualifiers
misc_feature            1..44
                        note = Primer
source                  1..44
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 411
aatgtacagt attgcgtttt gcgtcacgct tgaagaccac gttg                    44

SEQ ID NO: 412       moltype = DNA  length = 45
FEATURE              Location/Qualifiers
misc_feature         1..45
                     note = Primer
source               1..45
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 412
aatgtacagt attgcgtttt ggccagcatg cagttctaag gctct                   45

SEQ ID NO: 413       moltype = DNA  length = 43
FEATURE              Location/Qualifiers
misc_feature         1..43
                     note = Primer
source               1..43
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 413
aatgtacagt attgcgtttt ggtgcccgtc tcgactctta ggc                     43

SEQ ID NO: 414       moltype = DNA  length = 49
FEATURE              Location/Qualifiers
misc_feature         1..49
                     note = Primer
source               1..49
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 414
aatgtacagt attgcgtttt gtgtagccgc tgatcgtcgt gtatatgtc               49

SEQ ID NO: 415       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 415
aatgtacagt attgcgtttt ggactggtac tggttagtaa aggttgataa tattcca      57

SEQ ID NO: 416       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 416
aatgtacagt attgcgtttt gggtgaagta atcagtttgt tcactagtta cgtgatt      57

SEQ ID NO: 417       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 417
aatgtacagt attgcgtttt gctgacatgc ctactgatta ttcttcaaac tcatcac      57

SEQ ID NO: 418       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 418
aatgtacagt attgcgtttt gtgtgtgttt taattgttcc acttgagatt cttaacc      57

SEQ ID NO: 419       moltype = DNA  length = 57
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```
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 419
aatgtacagt attgcgtttt gcgtcagcat tttgaatcac ttcattctga catgata     57

SEQ ID NO: 420        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 420
aatgtacagt attgcgtttt gagtaatttt caactattgg cctagtgaat ttaagct     57

SEQ ID NO: 421        moltype = DNA  length = 55
FEATURE               Location/Qualifiers
misc_feature          1..55
                      note = Primer
source                1..55
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 421
aatgtacagt attgcgtttt gagaaagagg gaagtcacat ttatagagtg ctagc       55

SEQ ID NO: 422        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 422
aatgtacagt attgcgtttt gcatcaacag aaacagaaca acaaactgtg acaaatc     57

SEQ ID NO: 423        moltype = DNA  length = 57
FEATURE               Location/Qualifiers
misc_feature          1..57
                      note = Primer
source                1..57
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 423
aatgtacagt attgcgtttt gccaaagaat atccctttat atagcagtgg aacaatt     57

SEQ ID NO: 424        moltype = DNA  length = 55
FEATURE               Location/Qualifiers
misc_feature          1..55
                      note = Primer
source                1..55
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 424
aatgtacagt attgcgtttt gcagaatatg cagtgataag tgctgtttca tcact       55

SEQ ID NO: 425        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
SEQUENCE: 425
aatgtacagt attgcgtttt gttccccctg tgacgactac ttttcctc               48

SEQ ID NO: 426        moltype = DNA  length = 48
FEATURE               Location/Qualifiers
misc_feature          1..48
                      note = Primer
source                1..48
                      mol_type = other DNA
                      organism = synthetic construct
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SEQUENCE: 426
aatgtacagt attgcgtttt gcggtcccta tttcttcctc tgcttcgt              48

SEQ ID NO: 427         moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 427
aatgtacagt attgcgtttt gctgaacagt tctgtctcta ttacccgacc tc          52

SEQ ID NO: 428         moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 428
aatgtacagt attgcgtttt gcgttcatag ccttctatcc gagtatgtag ca          52

SEQ ID NO: 429         moltype = DNA  length = 47
FEATURE                Location/Qualifiers
misc_feature           1..47
                       note = Primer
source                 1..47
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 429
aatgtacagt attgcgtttt gccccttctg tcctcgcagg ttaatcc               47

SEQ ID NO: 430         moltype = DNA  length = 53
FEATURE                Location/Qualifiers
misc_feature           1..53
                       note = Primer
source                 1..53
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 430
aatgtacagt attgcgtttt ggcttccagc catttctgag atatcctcac agt         53

SEQ ID NO: 431         moltype = DNA  length = 52
FEATURE                Location/Qualifiers
misc_feature           1..52
                       note = Primer
source                 1..52
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 431
aatgtacagt attgcgtttt gaccaggagg aacaaagaca catgaagatc at          52

SEQ ID NO: 432         moltype = DNA  length = 45
FEATURE                Location/Qualifiers
misc_feature           1..45
                       note = Primer
source                 1..45
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 432
aatgtacagt attgcgtttt ggcgcccccg agtttcttac gaatc                  45

SEQ ID NO: 433         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
                       note = Primer
source                 1..57
                       mol_type = other DNA
                       organism = synthetic construct
SEQUENCE: 433
aatgtacagt attgcgtttt gtttatacac agtttggagt ttgagaatca gaagact      57

SEQ ID NO: 434         moltype = DNA  length = 57
FEATURE                Location/Qualifiers
misc_feature           1..57
```

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                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 434
aatgtacagt attgcgtttt gggttatctc tggctgatga gattatgagt gattctc     57

SEQ ID NO: 435       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 435
aatgtacagt attgcgtttt ggccaagcta gtgattgatg tgattcgcta t           51

SEQ ID NO: 436       moltype = DNA  length = 47
FEATURE              Location/Qualifiers
misc_feature         1..47
                     note = Primer
source               1..47
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 436
aatgtacagt attgcgtttt gcccctcctc tagtactccc tgtttgt                47

SEQ ID NO: 437       moltype = DNA  length = 51
FEATURE              Location/Qualifiers
misc_feature         1..51
                     note = Primer
source               1..51
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 437
aatgtacagt attgcgtttt gctccttcct gtcccaatca actagtctag c           51

SEQ ID NO: 438       moltype = DNA  length = 46
FEATURE              Location/Qualifiers
misc_feature         1..46
                     note = Primer
source               1..46
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 438
aatgtacagt attgcgtttt ggcctcgtcc ctcttccctt aggtaa                 46

SEQ ID NO: 439       moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 439
aatgtacagt attgcgtttt gtctctcttc ccattagtct gagtactgag tgatt        55

SEQ ID NO: 440       moltype = DNA  length = 56
FEATURE              Location/Qualifiers
misc_feature         1..56
                     note = Primer
source               1..56
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 440
aatgtacagt attgcgtttt gagcatttct tgagacttaa agtggcattc taaagg       56

SEQ ID NO: 441       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 441
aatgtacagt attgcgtttt gatttttatt ctcaagaggc agaaatacca acttacc      57
```

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SEQ ID NO: 442          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 442
aatgtacagt attgcgtttt gaatttatag ctcttttcat ctgctttggt atcatca     57

SEQ ID NO: 443          moltype = DNA  length = 56
FEATURE                 Location/Qualifiers
misc_feature            1..56
                        note = Primer
source                  1..56
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 443
aatgtacagt attgcgtttt ggcctctaat ctgatataca gccttagaaa gtcaca      56

SEQ ID NO: 444          moltype = DNA  length = 47
FEATURE                 Location/Qualifiers
misc_feature            1..47
                        note = Primer
source                  1..47
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 444
aatgtacagt attgcgtttt gtgtgccatt gtcctggagc aacaatt                47

SEQ ID NO: 445          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 445
aatgtacagt attgcgtttt gagtgtactg ctcgttttct taatttgaaa agtgagt     57

SEQ ID NO: 446          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 446
aatgtacagt attgcgtttt gacccatgaa ctaatactta ttttgagatt ggtccat     57

SEQ ID NO: 447          moltype = DNA  length = 55
FEATURE                 Location/Qualifiers
misc_feature            1..55
                        note = Primer
source                  1..55
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 447
aatgtacagt attgcgtttt gcatggtgca acaaaagtaa gaatccaaca gtttt       55

SEQ ID NO: 448          moltype = DNA  length = 57
FEATURE                 Location/Qualifiers
misc_feature            1..57
                        note = Primer
source                  1..57
                        mol_type = other DNA
                        organism = synthetic construct
SEQUENCE: 448
aatgtacagt attgcgtttt gttgaaatgt taagtaagct tgaaataccg atagcat     57

SEQ ID NO: 449          moltype = DNA  length = 51
FEATURE                 Location/Qualifiers
misc_feature            1..51
                        note = Primer
source                  1..51
```

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                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 449
aatgtacagt attgcgtttt ggggaggaag aaaatgaagc acgaggaaaa c          51

SEQ ID NO: 450       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 450
aatgtacagt attgcgtttt gatttgggat gtactctaaa tttaaagcag caaatca     57

SEQ ID NO: 451       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 451
aatgtacagt attgcgtttt gtcaagagca gaatttggag actttgatat taaaact     57

SEQ ID NO: 452       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 452
aatgtacagt attgcgtttt gcggttacta acatgtttag ggaaatagac aactgtt     57

SEQ ID NO: 453       moltype = DNA  length = 57
FEATURE              Location/Qualifiers
misc_feature         1..57
                     note = Primer
source               1..57
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 453
aatgtacagt attgcgtttt gcctgacaac agatcccata taattaactt tcatacc     57

SEQ ID NO: 454       moltype = DNA  length = 55
FEATURE              Location/Qualifiers
misc_feature         1..55
                     note = Primer
source               1..55
                     mol_type = other DNA
                     organism = synthetic construct
SEQUENCE: 454
aatgtacagt attgcgtttt gagatgaaga agatgaggaa cgagagagta aaagc       55
```

1.-**20**. (canceled)

21. A DNA tag comprising a unique molecular identifier (UMI) and a DNA identifier, wherein the DNA tag further comprises a single-stranded portion at a 3' end.

22. The DNA tag of claim **21**, wherein the UMI and the DNA identifier are positioned in a 5' to 3' direction.

23. A RNA tag comprising a RNA identifier, a UMI, and a poly(T) or a template switching oligonucleotide (TSO).

24. The RNA tag of claim **23**, wherein the RNA identifier, the UMI, and the poly(T) are positioned in a 5' to 3' direction.

25. (canceled)

26. The RNA tag of claim **23**, wherein the RNA identifier, the UMI, and the TSO are positioned in a 5' to 3' direction.

27.-**29**. (canceled)

30. A composition comprising a DNA tag comprising a unique molecular identifier (UMI) and a DNA identifier, and a RNA comprising a RNA identifier, a UMI, and a poly(T).

31. The composition of claim **30**, wherein the DNA tag comprises the UMI and the DNA identifier positioned in a 5' to 3' direction.

32. The composition of claim **30**, wherein the RNA tag comprises the RNA identifier, the UMI, and the poly(T) positioned in a 5' to 3' position.

33. The composition of claim **30**, further comprising a RNA tag comprising a RNA identifier, a UMI, and a template switching oligonucleotide (TSO).

34. The composition of claim **30**, further comprising a set of gene specific primers.

35. The composition of claim **30**, further comprising primers specific for the DNA tag.

36. The composition of claim **30**, further comprising primers specific for the RNA tag.

37. The composition of claim **30**, wherein the DNA tag further comprises a single-stranded portion at a 3' end.

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