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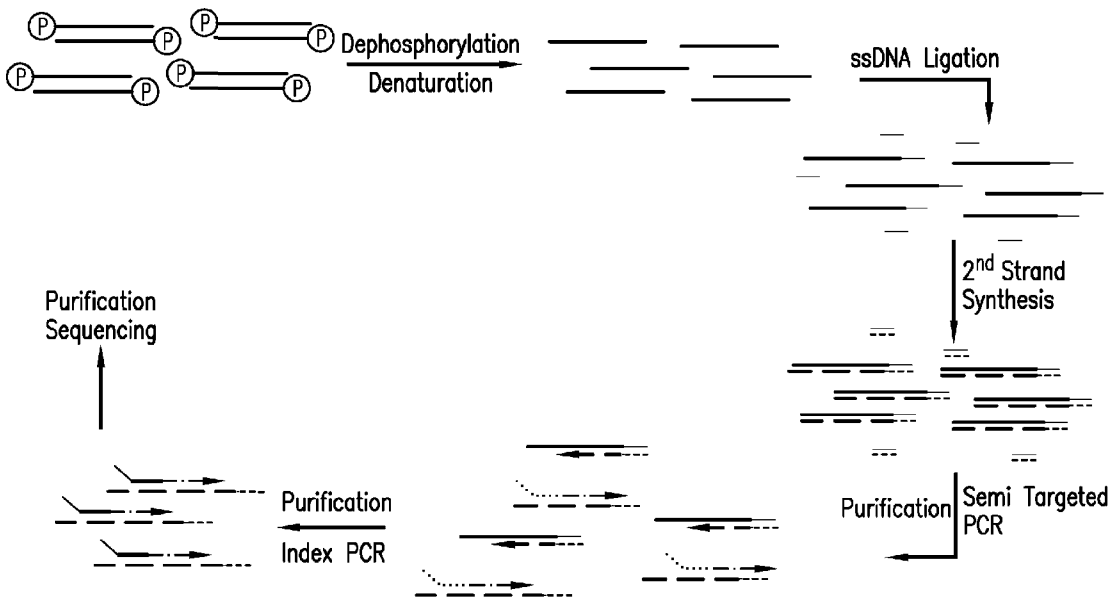


FIG. 1

(57) Abstract: The present disclosure relates to methods for constructing polynucleotide libraries and/or polynucleotide sequencing. Related kits and devices are also disclosed. The present disclosure also relates to compositions, kits, devices, and methods for conducting genetic and genomic analysis, for example, by polynucleotide sequencing. In particular aspects, provided herein are compositions, kits, and methods for constructing libraries with improved ligation efficiency and conversion rate during sequencing. In certain embodiments, the compositions, kits, and methods herein are useful for analyzing polynucleotide fragments, such as circulating polynucleotide fragments in the body of a subject, Including circulating tumor DNA

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COMPOSITIONS AND METHODS FOR LIBRARY CONSTRUCTION AND SEQUENCE ANALYSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of priority to U.S. Provisional Application Serial No. 62/487,423, filed April 19, 2017, and U.S. Provisional Application Serial No. 62/657,544, filed April 13, 2018, the contents of both applications are incorporated by reference in their entireties for all purposes. In some aspect, the present disclosure relates to U.S. Provisional Application Serial No. 62/487,422, filed on April 19, 2017, the content of which is incorporated by reference its entirety for all purposes.

TECHNICAL FIELD

[0002] The present disclosure relates to compositions, kits, devices, and methods for conducting genetic and genomic analysis, for example, by polynucleotide sequencing. In particular aspects, provided herein are compositions, kits, and methods for constructing libraries with improved ligation efficiency and conversion rate during sequencing. In certain embodiments, the compositions, kits, and methods herein are useful for analyzing polynucleotide fragments, such as circulating polynucleotide fragments in the body of a subject, including circulating tumor DNA.

BACKGROUND

[0003] In the following discussion, certain articles and methods are described for background and introductory purposes. Nothing contained herein is to be construed as an "admission" of prior art. Applicant expressly reserves the right to demonstrate, where appropriate, that the articles and methods referenced herein do not constitute prior art under the applicable statutory provisions.

[0004] Despite several improvements in library construction over the last several years, the process of library construction for next generation sequencing remains inefficient, resulting in many original molecules lost during the various steps. Double stranded ligation efficiency remains low, with ~20-30% of the molecules being properly ligated. Additionally, many molecules are lost during the purification and hybridization capture steps, so that the final conversion rate approximates 10-20%. Sensitivity remains low when interrogating low allele

fraction variants, for example, those found in circulating tumor DNA (ctDNA). This limits the accuracy when calling low allele fraction mutants, since the low efficiency will result in sensitivity loss when looking at libraries with low allele fractions.

[0005] There is a need for improved analytical technology to overcome the above issues of art. The present disclosure addresses this and other related needs.

BRIEF SUMMARY

[0006] The summary is not intended to be used to limit the scope of the claimed subject matter. Other features, details, utilities, and advantages of the claimed subject matter will be apparent from the detailed description including those aspects disclosed in the accompanying drawings and in the appended claims.

[0007] In one embodiment, provided herein is a method, comprising ligating a set of adaptors to a library of single-stranded polynucleotides. In one aspect, the ligation is catalyzed by a single-stranded DNA (ssDNA) ligase. In another aspect, each single-stranded polynucleotide is blocked at the 5' end to prevent ligation at the 5' end. In yet another aspect, each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide to which the adaptor is ligated. In one other aspect, each adaptor is blocked at the 3' end to prevent ligation at the 3' end. In one aspect, the 5' end of the adaptor is ligated to the 3' end of the single-stranded polynucleotide by the ssDNA ligase to form a linear ligation product. In any of the preceding embodiments, a library of linear, single-stranded ligation products can be obtained.

[0008] In another embodiment, a method comprising ligating a set of adaptors to a library of single-stranded polynucleotides is provided, and in the method, the ligation is catalyzed by a single-stranded DNA (ssDNA) ligase, each single-stranded polynucleotide is blocked at the 5' end to prevent ligation at the 5' end, each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide to which the adaptor is ligated, each adaptor is blocked at the 3' end to prevent ligation at the 3' end, and the 5' end of the adaptor is ligated to the 3' end of the single-stranded polynucleotide by the ssDNA ligase to form a linear ligation product, thereby obtaining a library of linear, single-stranded ligation products.

[0009] In any of the preceding embodiments, the method can further comprise before the ligation step, a step of obtaining the library of single-stranded polynucleotides from a sample. In one aspect, the obtaining step comprises denaturing double-stranded polynucleotides from the sample.

[0010] In any of the preceding embodiments, the sample can be a biological sample. In some embodiments, the biological sample is obtained directly from a subject without any treatment. In some embodiments, the polynucleotides in the biological sample have not been subject to bisulfite conversion. In other embodiments, the polynucleotides in the biological sample have been subject to partial or complete bisulfite conversion. In certain aspects, the biological sample is from a subject having or suspected of having a disease or condition, such as a cancer or neoplasia.

[0011] In any of the preceding embodiments, the single-stranded polynucleotides can be from a sample comprising circulating tumor DNA (ctDNA), such as a blood, serum, plasma, or body fluid sample, or any combination thereof.

[0012] In any of the preceding embodiments, the single-stranded polynucleotides can be between about 20 and about 400 nucleic acid residues in length, for example, about 80, about 100, about 120, about 140, about 160, about 180, about 200, about 220, or about 240 nucleic acid residues in length.

[0013] In any of the preceding embodiments, the ssDNA ligase can be a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), or an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1. In other aspects, the ssDNA ligase is an RNA ligase, such as a T4 RNA ligase, *e.g.*, T4 RNA ligase I, *e.g.*, New England Biosciences, M0204S, T4 RNA ligase 2, *e.g.*, New England Biosciences, M0239S, T4 RNA ligase 2 truncated, *e.g.*, New England Biosciences, M0242S, T4 RNA ligase 2 truncated KQ, *e.g.*, M0373S, or T4 RNA ligase 2 truncated K227Q, *e.g.*, New England Biosciences, M0351S. In any of the preceding embodiments, the kit can also comprise a thermostable 5' App DNA/RNA ligase, *e.g.*, New England Biosciences, M0319S, or T4 DNA ligase, *e.g.*, New England Biosciences, M0202S.

[0014] In any of the preceding embodiments, the blocking of each single-stranded polynucleotide can comprise dephosphorylation to prevent ligation at its 5' end.

[0015] In any of the preceding embodiments, the blocking of each adaptor can comprise a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol, and/or hexaethylene glycol, to prevent ligation at its 3' end.

[0016] In any of the preceding embodiments, each adaptor can comprise a dinucleotide sequence at the 5' end, such as GA (5' to 3'), GG (5' to 3'), AA (5' to 3'), or AG (5' to 3'), which is 5' to the UMI sequence.

[0017] In any of the preceding embodiments, the UMI sequence in each adaptor can be between about 6 and about 15 nucleic acid residues in length, for example, the UMI sequence is a 12-mer.

[0018] In any of the preceding embodiments, the ligation reaction can be conducted in the presence of a crowding agent. In one aspect, the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000 or PEG 6000, Dextran, and/or Ficoll.

[0019] In any of the preceding embodiments, the method can further comprise converting the library of linear, single-stranded ligation products into a library of linear, double-stranded ligation products. In one aspect, the conversion uses a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor.

[0020] In any of the preceding embodiments, the method can further comprise amplifying and/or purifying the library of linear, double-stranded ligation products. In one aspect, the purification is bead-based. In another aspect, the purification is based on size selection, for example, the purification step selectively purifies polynucleotides between about 50 nucleotides and about 1000 nucleotides in lengths, for example, adaptors of about 40 nucleotides in length (and primer dimers and/or primer-adaptor duplexes of about 40bp) are removed. In another aspect, the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead). In one aspect, the purification is column-based, for example, by using a dsDNA or ssDNA purification column, such as those from Zymo or Qiagen.

[0021] In any of the preceding embodiments, the method herein can further comprise amplifying the library of linear, double-stranded ligation products, *e.g.*, by a polymerase chain reaction (PCR), to obtain an amplified library of linear, double-stranded ligation products

comprising sequence information of a target sequence. In one aspect, the method comprises using a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor. In another aspect, the method further comprises using a primer hybridizable to the target sequence (*e.g.*, an EGFR gene sequence).

[0022] In any of the preceding embodiments, the method herein can comprise amplifying the library of linear, double-stranded ligation products, *e.g.*, by a polymerase chain reaction (PCR), using a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor, a primer hybridizable to the target sequence (*e.g.*, an EGFR gene sequence), thereby obtaining an amplified library of linear, double-stranded ligation products comprising sequence information of the target sequence.

[0023] In any of the preceding embodiments, the target-specific primer can comprise any one or more sequences selected from the group consisting of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof.

[0024] In any of the preceding embodiments, a plurality of primers can be used, each comprising a sequence specific for the target sequence and the primers have the same or different target sequences. In one aspect, the plurality of primers comprise any one or more, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

[0025] In any of the preceding embodiments, the sequence information of the target sequence can comprise a mutation, a single nucleotide polymorphism (SNP), a copy number variation (CNV), or an epigenetic change. In one aspect, the mutation comprises a point mutation, an insertion, a deletion, an inversion, a truncation, a fusion, an amplification, or any combination thereof.

[0026] In any of the preceding embodiments, the amplified library of linear, double-stranded ligation products can be a library other than a whole genome library, for example, a semi-targeted genome library.

[0027] In any of the preceding embodiments, the method can further comprise purifying the amplified library of linear, double-stranded ligation products. In one aspect, the purification is bead-based. In another aspect, the purification is based on size selection, for example, the purification step selectively purifies polynucleotides greater about 150 nucleotides in lengths. In

another aspect, the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead). In one aspect, the purification is column-based, for example, by using a dsDNA or ssDNA purification column, such as those from Zymo or Qiagen.

[0028] In any of the preceding embodiments, the method can further comprise sequencing the purified amplified library of linear, double-stranded ligation products. In one aspect, the sequencing step comprises attaching a sequencing adapter and/or a sample-specific barcode to each linear, double-stranded ligation product. In one particular aspect, the attaching step is performed using a polymerase chain reaction (PCR).

[0029] In any of the preceding embodiments, the conversion rate of the sequencing (percentage of single-stranded polynucleotides in the library that give rise to sequencing reads) may be at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

[0030] In any of the preceding embodiments, the method can be used for the diagnosis and/or prognosis of a disease or condition in a subject, predicting the responsiveness of a subject to a treatment, identifying a pharmacogenetics marker for the disease/condition or treatment, and/or screening a population for a genetic information. In one aspect, the disease or condition is a cancer or neoplasia, and the treatment is a cancer or neoplasia treatment.

[0031] In another aspect, disclosed herein is a library of linear, single-stranded ligation products produced by the method of any of proceeding embodiments.

[0032] In yet another aspect, disclosed herein is a library of linear, double-stranded ligation products produced by the method of any of proceeding embodiments.

[0033] In still another aspect, disclosed herein is an amplified library of linear, double-stranded ligation products produced by the method of any of proceeding embodiments.

[0034] In one other aspect, disclosed herein is a sequencing library produced by the method of any of proceeding embodiments.

[0035] Disclosed in another aspect herein is a kit for constructing a library of ligation products. In one embodiment, the kit comprises a single-stranded DNA (ssDNA) ligase. In another aspect, the kit comprises a plurality of adaptors. In particular aspects, each adaptor is blocked to prevent ligation at the 3' end while the 5' end of the adaptor is available for ligation

to a single-stranded polynucleotide to form a linear, single-stranded ligation product. In further particular aspects, each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide.

[0036] In any of the preceding embodiments, the kit for constructing a library of ligation products can comprise a ssDNA ligase and a plurality of adaptors, and each adaptor is blocked to prevent ligation at the 3' end while the 5' end of the adaptor is available for ligation to a single-stranded polynucleotide to form a linear, single-stranded ligation product, and each adaptor comprises a UMI sequence that earmarks the single-stranded polynucleotide.

[0037] In any of the preceding embodiments, the kit can further comprise a denaturing reagent for denaturing a double-stranded polynucleotide from a sample to obtain the single-stranded polynucleotide.

[0038] In any of the preceding embodiments, the kit can comprise a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), or an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1. In any of the preceding embodiments, the kit can comprise an RNA ligase, such as a T4 RNA ligase, *e.g.*, T4 RNA ligase I, *e.g.*, New England Biosciences, M0204S, T4 RNA ligase 2, *e.g.*, New England Biosciences, M0239S, T4 RNA ligase 2 truncated, *e.g.*, New England Biosciences, M0242S, T4 RNA ligase 2 truncated KQ, *e.g.*, M0373S, or T4 RNA ligase 2 truncated K227Q, *e.g.*, New England Biosciences, M0351S. In any of the preceding embodiments, the kit can also comprise a thermostable 5' App DNA/RNA ligase, *e.g.*, New England Biosciences, M0319S, or T4 DNA ligase, *e.g.*, New England Biosciences, M0202S.

[0039] In any of the preceding embodiments, the kit can further comprise a dephosphorylating reagent for removing a 5' phosphate group of the single-stranded polynucleotide. In any of the preceding embodiments, the blocking of each adaptor can comprise a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol, and/or hexaethylene glycol, to prevent ligation at its 3' end. In any of the preceding embodiments of the kit, each adaptor can comprise a dinucleotide sequence at the 5' end, such as GA (5' to 3'), GG (5' to 3'), AA (5' to 3'), or AG (5' to 3'). In any of the preceding embodiments, the UMI sequence in each adaptor can be between about 6 and about 15 nucleic acid residues in length, for example, the UMI sequence is a 12-mer.

[0040] In any of the preceding embodiments, the kit can further comprise a crowding agent for the ligation reaction. In one aspect, the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000 or PEG 6000, Dextran, and/or Ficoll.

[0041] In any of the preceding embodiments, the kit can further comprise a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor, for converting the single-stranded polynucleotide to a double-stranded polynucleotide.

[0042] In any of the preceding embodiments, the kit can further comprise a reagent for removing primer dimer and/or primer-adaptor duplex.

[0043] In any of the preceding embodiments, the kit can further comprise a primer comprising a sequence specific for a target sequence (*e.g.*, an EGFR gene sequence), for obtaining an amplified linear, double-stranded ligation product comprising sequence information of the target sequence. In any of the preceding embodiments, the target-specific primer can comprise any one or more sequences selected from the group consisting of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof.

[0044] In any of the preceding embodiments, the kit can comprise a plurality of primers, each comprising a sequence specific for the target sequence, wherein the primers have the same or different target sequences. In one aspect, the plurality of primers comprise any one or more, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

[0045] In any of the preceding embodiments, the kit can further comprise a sequencing adapter and/or a sample-specific barcode, for sequencing the amplified linear, double-stranded ligation product.

[0046] In any of the preceding embodiments, the kit can further comprise separate vials for each component and/or instructions for using the components. In one aspect, the instructions comprise obtaining the single-stranded polynucleotide from a sample that comprises circulating tumor DNA (ctDNA), such as a blood, serum, plasma, or body fluid sample, or any combination thereof.

[0047] Also disclosed herein is a polynucleotide comprising AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 1) or a portion thereof, *e.g.*, a portion that comprises between about 18 and 22 nucleotide residues.

[0048] In one aspect, disclosed herein is a polynucleotide comprising $N_1 \ldots N_i$AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG or a portion thereof, wherein N_1 to N_i is any nucleic acid residue, for example, A, T, C, or G, and *i* is an integer between about 4 and about 25.

[0049] In another aspect, disclosed herein is a polynucleotide comprising GANNNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 2) or a portion thereof, *e.g.*, a portion that comprises between about 32 and 36 nucleotide residues, wherein N is any nucleic acid residue, for example, A, T, C, or G.

[0050] In one aspect, disclosed herein is a polynucleotide comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof, *e.g.*, a portion that comprises between about 12 and 20 nucleotide residues.

[0051] In one other aspect, disclosed herein is a primer comprising any one or more sequences selected from the group consisting of SEQ ID NOs: 4-1529. In one aspect, disclosed herein is a primer set comprising any one or more, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof. In one aspect, disclosed herein is a primer set comprising any one or more, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof, and a primer comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof. In one other aspect, disclosed herein is a kit comprising any one or more, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof, a primer comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof, and/or a polynucleotide comprising AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 1) or a portion thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] **FIG. 1** shows steps for constructing a single-stranded polynucleotide library and performing sequencing analysis using the library, according to one aspect of the present disclosure.

[0053] **FIG. 2** shows a construct comprising a target molecule for sequencing, according to one aspect of the present disclosure.

[0054] **FIG. 3** shows the linear correlation between the expected allele fraction and the observed allele fraction using a method disclosed herein, indicating that the method has great reproducibility.

[0055] **FIG. 4** compares the allele fraction for multiple variants before and after error-correction, using a method disclosed herein.

[0056] **FIG. 5** compares the performance parameters of a method disclosed herein and the conventional hybridization capture method for library construction and sequencing.

[0057] **FIG. 6** compares the conversion rates of a method disclosed herein (TitanSeq) and the conventional hybridization capture method.

[0058] **FIG. 7** shows additional exemplary primer(s) or primer pool(s).

DETAILED DESCRIPTION

[0059] Numerous specific details are set forth in the following description in order to provide a thorough understanding of the present disclosure. These details are provided for the purpose of example and the claimed subject matter may be practiced according to the claims without some or all of these specific details. It is to be understood that other embodiments can be used and structural changes can be made without departing from the scope of the claimed subject matter. It should be understood that the various features and functionality described in one or more of the individual embodiments are not limited in their applicability to the particular embodiment with which they are described. They instead can, be applied, alone or in some combination, to one or more of the other embodiments of the disclosure, whether or not such embodiments are described, and whether or not such features are presented as being a part of a described embodiment. For the purpose of clarity, technical material that is known in the technical fields related to the claimed subject matter has not been described in detail so that the claimed subject matter is not unnecessarily obscured.

[0060] All publications, including patent documents, scientific articles and databases, referred to in this application are incorporated by reference in their entireties for all purposes to the same extent as if each individual publication were individually incorporated by reference. Citation of the publications or documents is not intended as an admission that any of them is pertinent prior art, nor does it constitute any admission as to the contents or date of these publications or documents.

[0061] All headings are for the convenience of the reader and should not be used to limit the meaning of the text that follows the heading, unless so specified.

[0062] The practice of the provided embodiments will employ, unless otherwise indicated, conventional techniques and descriptions of organic chemistry, polymer technology, molecular biology (including recombinant techniques), cell biology, biochemistry, and sequencing technology, which are within the skill of those who practice in the art. Such conventional techniques include polypeptide and protein synthesis and modification, polynucleotide synthesis and modification, polymer array synthesis, hybridization and ligation of polynucleotides, detection of hybridization, and nucleotide sequencing. Specific illustrations of suitable techniques can be had by reference to the examples herein. However, other equivalent conventional procedures can, of course, also be used. Such conventional techniques and descriptions can be found in standard laboratory manuals such as Green, *et al.*, Eds., *Genome Analysis: A Laboratory Manual Series* (Vols. I-IV) (1999); Weiner, Gabriel, Stephens, Eds., *Genetic Variation: A Laboratory Manual* (2007); Dieffenbach, Dveksler, Eds., *PCR Primer: A Laboratory Manual* (2003); Bowtell and Sambrook, *DNA Microarrays: A Molecular Cloning Manual* (2003); Mount, *Bioinformatics: Sequence and Genome Analysis* (2004); Sambrook and Russell, *Condensed Protocols from Molecular Cloning: A Laboratory Manual* (2006); and Sambrook and Russell, *Molecular Cloning: A Laboratory Manual* (2002) (all from Cold Spring Harbor Laboratory Press); Ausubel *et al.* eds., *Current Protocols in Molecular Biology* (1987); T. Brown ed., *Essential Molecular Biology* (1991), IRL Press; Goeddel ed., *Gene Expression Technology* (1991), Academic Press; A. Bothwell *et al.* eds., *Methods for Cloning and Analysis of Eukaryotic Genes* (1990), Bartlett Publ.; M. Kriegler, *Gene Transfer and Expression* (1990), Stockton Press; R. Wu *et al.* eds., *Recombinant DNA Methodology* (1989), Academic Press; M. McPherson *et al.*, *PCR: A Practical Approach* (1991), IRL Press at Oxford University Press; Stryer, *Biochemistry* (4th Ed.) (1995), W. H. Freeman, New York N.Y.; Gait, *Oligonucleotide*

Synthesis: A Practical Approach (2002), IRL Press, London; Nelson and Cox, *Lehninger, Principles of Biochemistry* (2000) *3rd* Ed., W. H. Freeman Pub., New York, N.Y.; Berg, *et al.*, *Biochemistry* (2002) *5th* Ed., W. H. Freeman Pub., New York, N.Y., all of which are herein incorporated in their entireties by reference for all purposes.

A. Definitions

[0063] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art to which the present disclosure belongs. If a definition set forth in this section is contrary to or otherwise inconsistent with a definition set forth in the patents, applications, published applications and other publications that are herein incorporated by reference, the definition set forth in this section prevails over the definition that is incorporated herein by reference.

[0064] As used herein, "a" or "an" means "at least one" or "one or more." As used herein, the singular forms "a," "an," and "the" include the plural reference unless the context clearly dictates otherwise.

[0065] Throughout this disclosure, various aspects of the claimed subject matter are presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the claimed subject matter. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, where a range of values is provided, it is understood that each intervening value, between the upper and lower limit of that range and any other stated or intervening value in that stated range is encompassed within the claimed subject matter. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the claimed subject matter, subject to any specifically excluded limit in the stated range. Where the stated range includes one or both of the limits, ranges excluding either or both of those included limits are also included in the claimed subject matter. This applies regardless of the breadth of the range.

[0066] Reference to "about" a value or parameter herein includes (and describes) variations that are directed to that value or parameter per se. For example, description referring to "about X" includes description of "X". Additionally, use of "about" preceding any series of numbers

includes "about" each of the recited numbers in that series. For example, description referring to "about X, Y, or Z" is intended to describe "about X, about Y, or about Z."

[0067] The term "average" as used herein refers to either a mean or a median, or any value used to approximate the mean or the median, unless the context clearly indicates otherwise.

[0068] A "subject" as used herein refers to an organism, or a part or component of the organism, to which the provided compositions, methods, kits, devices, and systems can be administered or applied. For example, the subject can be a mammal or a cell, a tissue, an organ, or a part of the mammal. As used herein, "mammal" refers to any of the mammalian class of species, preferably human (including humans, human subjects, or human patients). Mammals include, but are not limited to, farm animals, sport animals, pets, primates, horses, dogs, cats, and rodents such as mice and rats.

[0069] As used herein the term "sample" refers to anything which may contain a target molecule for which analysis is desired, including a biological sample. As used herein, a "biological sample" can refer to any sample obtained from a living or viral (or prion) source or other source of macromolecules and biomolecules, and includes any cell type or tissue of a subject from which nucleic acid, protein and/or other macromolecule can be obtained. The biological sample can be a sample obtained directly from a biological source or a sample that is processed. For example, isolated nucleic acids that are amplified constitute a biological sample. Biological samples include, but are not limited to, body fluids, such as blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine, sweat, semen, stool, sputum, tears, mucus, amniotic fluid or the like, an effusion, a bone marrow sample, ascitic fluid, pelvic wash fluid, pleural fluid, spinal fluid, lymph, ocular fluid, extract of nasal, throat or genital swab, cell suspension from digested tissue, or extract of fecal material, and tissue and organ samples from animals and plants and processed samples derived therefrom.

[0070] The terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" are used interchangeably herein to refer to a polymeric form of nucleotides of any length, and comprise ribonucleotides, deoxyribonucleotides, and analogs or mixtures thereof. The terms include triple-, double- and single-stranded deoxyribonucleic acid ("DNA"), as well as triple-, double- and single-stranded ribonucleic acid ("RNA"). It also includes modified, for example by alkylation, and/or by capping, and unmodified forms of the polynucleotide. More particularly, the terms "polynucleotide," "oligonucleotide," "nucleic acid," and "nucleic acid

molecule" include polydeoxyribonucleotides (containing 2-deoxy-D-ribose), polyribonucleotides (containing D-ribose), including tRNA, rRNA, hRNA, and mRNA, whether spliced or unspliced, any other type of polynucleotide which is an N- or C-glycoside of a purine or pyrimidine base, and other polymers containing nonnucleotidic backbones, for example, polyamide (*e.g.*, peptide nucleic acids ("PNAs")) and polymorpholino (commercially available from the Anti-Virals, Inc., Corvallis, OR, as Neugene) polymers, and other synthetic sequence-specific nucleic acid polymers providing that the polymers contain nucleobases in a configuration which allows for base pairing and base stacking, such as is found in DNA and RNA. Thus, these terms include, for example, 3'-deoxy-2',5'-DNA, oligodeoxyribonucleotide N3' to P5' phosphoramidates, 2'-O-alkyl-substituted RNA, hybrids between DNA and RNA or between PNAs and DNA or RNA, and also include known types of modifications, for example, labels, alkylation, "caps," substitution of one or more of the nucleotides with an analog, inter-nucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, *etc.*), with negatively charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), and with positively charged linkages (*e.g.*, aminoalkylphosphoramidates, aminoalkylphosphotriesters), those containing pendant moieties, such as, for example, proteins (including enzymes (*e.g.* nucleases), toxins, antibodies, signal peptides, poly-L-lysine, *etc.*), those with intercalators (*e.g.*, acridine, psoralen, *etc.*), those containing chelates (of, *e.g.*, metals, radioactive metals, boron, oxidative metals, *etc.*), those containing alkylators, those with modified linkages (*e.g.*, alpha anomeric nucleic acids, *etc.*), as well as unmodified forms of the polynucleotide or oligonucleotide. A nucleic acid generally will contain phosphodiester bonds, although in some cases nucleic acid analogs may be included that have alternative backbones such as phosphoramidite, phosphorodithioate, or methylphophoroamidite linkages; or peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with bicyclic structures including locked nucleic acids, positive backbones, non-ionic backbones and non-ribose backbones. Modifications of the ribose-phosphate backbone may be done to increase the stability of the molecules; for example, PNA:DNA hybrids can exhibit higher stability in some environments. The terms "polynucleotide," "oligonucleotide," "nucleic acid" and "nucleic acid molecule" can comprise any suitable length, such as at least 5, 6, 7, 8, 9, 10, 20, 30, 40, 50, 100, 200, 300, 400, 500, 1,000 or more nucleotides.

[0071] It will be appreciated that, as used herein, the terms "nucleoside" and "nucleotide" include those moieties which contain not only the known purine and pyrimidine bases, but also other heterocyclic bases which have been modified. Such modifications include methylated purines or pyrimidines, acylated purines or pyrimidines, or other heterocycles. Modified nucleosides or nucleotides can also include modifications on the sugar moiety, *e.g.*, wherein one or more of the hydroxyl groups are replaced with halogen, aliphatic groups, or are functionalized as ethers, amines, or the like. The term "nucleotidic unit" is intended to encompass nucleosides and nucleotides.

[0072] The terms "complementary" and "substantially complementary" include the hybridization or base pairing or the formation of a duplex between nucleotides or nucleic acids, for instance, between the two strands of a double-stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single-stranded nucleic acid. Complementary nucleotides are, generally, A and T (or A and U), or C and G. Two single-stranded RNA or DNA molecules are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared and with appropriate nucleotide insertions or deletions, pair with at least about 80% of the other strand, usually at least about 90% to about 95%, and even about 98% to about 100%. In one aspect, two complementary sequences of nucleotides are capable of hybridizing, preferably with less than 25%, more preferably with less than 15%, even more preferably with less than 5%, most preferably with no mismatches between opposed nucleotides. Preferably the two molecules will hybridize under conditions of high stringency.

[0073] As used herein, for a reference sequence, the reverse complementary sequence is the complementary sequence of the reference sequence in the reverse order. For example, for 5'-ATCG-3', the complementary sequence is 3'-TAGC-5', and the reverse-complementary sequence is 5'-CGAT-3'.

[0074] "Hybridization" as used herein may refer to the process in which two single-stranded polynucleotides bind non-covalently to form a stable double-stranded polynucleotide. In one aspect, the resulting double-stranded polynucleotide can be a "hybrid" or "duplex." "Hybridization conditions" typically include salt concentrations of approximately less than 1 M, often less than about 500 mM and may be less than about 200 mM. A "hybridization buffer" includes a buffered salt solution such as 5% SSPE, or other such buffers known in the art.

Hybridization temperatures can be as low as 5°C, but are typically greater than 22°C, and more typically greater than about 30°C, and typically in excess of 37°C. Hybridizations are often performed under stringent conditions, *i.e.*, conditions under which a sequence will hybridize to its target sequence but will not hybridize to other, non-complementary sequences. Stringent conditions are sequence-dependent and are different in different circumstances. For example, longer fragments may require higher hybridization temperatures for specific hybridization than short fragments. As other factors may affect the stringency of hybridization, including base composition and length of the complementary strands, presence of organic solvents, and the extent of base mismatching, the combination of parameters is more important than the absolute measure of any one parameter alone. Generally stringent conditions are selected to be about 5°C lower than the T_m for the specific sequence at a defined ionic strength and pH. The melting temperature T_m can be the temperature at which a population of double-stranded nucleic acid molecules becomes half dissociated into single strands. Several equations for calculating the T_m of nucleic acids are well known in the art. As indicated by standard references, a simple estimate of the T_m value may be calculated by the equation, T_m =81.5 + 0.41 (% G + C), when a nucleic acid is in aqueous solution at 1 M NaCl (*see e.g.*, Anderson and Young, Quantitative Filter Hybridization, in *Nucleic Acid Hybridization* (1985)). Other references (*e.g.*, Allawi and SantaLucia, Jr., *Biochemistry*, 36:10581-94 (1997)) include alternative methods of computation which take structural and environmental, as well as sequence characteristics into account for the calculation of T_m.

[0075] In general, the stability of a hybrid is a function of the ion concentration and temperature. Typically, a hybridization reaction is performed under conditions of lower stringency, followed by washes of varying, but higher, stringency. Exemplary stringent conditions include a salt concentration of at least 0.01 M to no more than 1 M sodium ion concentration (or other salt) at a pH of about 7.0 to about 8.3 and a temperature of at least 25°C. For example, conditions of 5 × SSPE (750 mM NaCl, 50 mM sodium phosphate, 5 mM EDTA at pH 7.4) and a temperature of approximately 30°C are suitable for allele-specific hybridizations, though a suitable temperature depends on the length and/or GC content of the region hybridized. In one aspect, "stringency of hybridization" in determining percentage mismatch can be as follows: 1) high stringency: 0.1 × SSPE, 0.1% SDS, 65°C; 2) medium stringency: 0.2 × SSPE, 0.1% SDS, 50°C (also referred to as moderate stringency); and 3) low

stringency: 1.0 × SSPE, 0.1% SDS, 50°C. It is understood that equivalent stringencies may be achieved using alternative buffers, salts and temperatures. For example, moderately stringent hybridization can refer to conditions that permit a nucleic acid molecule such as a probe to bind a complementary nucleic acid molecule. The hybridized nucleic acid molecules generally have at least 60% identity, including for example at least any of 70%, 75%, 80%, 85%, 90%, or 95% identity. Moderately stringent conditions can be conditions equivalent to hybridization in 50% formamide, 5 ⁄ Denhardt's solution, 5x SSPE, 0.2% SDS at 42°C, followed by washing in 0.2 × SSPE, 0.2% SDS, at 42°C. High stringency conditions can be provided, for example, by hybridization in 50% formamide, 5 × Denhardt's solution, 5 × SSPE, 0.2% SDS at 42°C, followed by washing in 0.1 × SSPE, and 0.1% SDS at 65°C. Low stringency hybridization can refer to conditions equivalent to hybridization in 10% formamide, 5 × Denhardt's solution, 6 × SSPE, 0.2% SDS at 22°C, followed by washing in 1x SSPE, 0.2% SDS, at 37°C. Denhardt's solution contains 1% Ficoll, 1% polyvinylpyrolidone, and 1% bovine serum albumin (BSA). 20 ⁄ SSPE (sodium chloride, sodium phosphate, EDTA) contains 3 M sodium chloride, 0.2 M sodium phosphate, and 0.025 M EDTA. Other suitable moderate stringency and high stringency hybridization buffers and conditions are well known to those of skill in the art and are described, for example, in Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press, Plainview, N.Y. (1989); and Ausubel *et al.*, Short Protocols in Molecular Biology, 4th ed., John Wiley & Sons (1999).

[0076] Alternatively, substantial complementarity exists when an RNA or DNA strand will hybridize under selective hybridization conditions to its complement. Typically, selective hybridization will occur when there is at least about 65% complementary over a stretch of at least 14 to 25 nucleotides, preferably at least about 75%, more preferably at least about 90% complementary. *See* M. Kanehisa, *Nucleic Acids Res.* 12.203 (1984).

[0077] A "primer" used herein can be 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. Primers usually are extended by a polymerase, for example, a DNA polymerase.

[0078] "Ligation" may refer to the formation of a covalent bond or linkage between the termini of two or more nucleic acids, *e.g.*, oligonucleotides and/or polynucleotides, in a template-driven reaction. The nature of the bond or linkage may vary widely and the ligation may be carried out enzymatically. As used herein, ligations are usually carried out enzymatically to form a phosphodiester linkage between a 5' carbon terminal nucleotide of one oligonucleotide with a 3' carbon of another nucleotide.

[0079] "Amplification," as used herein, generally refers to the process of producing multiple copies of a desired sequence. "Multiple copies" means at least 2 copies. A "copy" does not necessarily mean perfect sequence complementarity or identity to the template sequence. For example, copies can include nucleotide analogs such as deoxyinosine, intentional sequence alterations (such as sequence alterations introduced through a primer comprising a sequence that is hybridizable, but not complementary, to the template), and/or sequence errors that occur during amplification.

[0080] "Sequence determination" and the like include determination of information relating to the nucleotide base sequence of a nucleic acid. Such information may include the identification or determination of partial as well as full sequence information of the nucleic acid. Sequence information may be determined with varying degrees of statistical reliability or confidence. In one aspect, the term includes the determination of the identity and ordering of a plurality of contiguous nucleotides in a nucleic acid.

[0081] The term "Sequencing," "High throughput sequencing," or "next generation sequencing" includes sequence determination using methods that determine many (typically thousands to billions) of nucleic acid sequences in an intrinsically parallel manner, *i.e.* where DNA templates are prepared for sequencing not one at a time, but in a bulk process, and where many sequences are read out preferably in parallel, or alternatively using an ultra-high throughput serial process that itself may be parallelized. Such methods include but are not limited to pyrosequencing (for example, as commercialized by 454 Life Sciences, Inc., Branford, CT); sequencing by ligation (for example, as commercialized in the SOLiD™ technology, Life Technologies, Inc., Carlsbad, CA); sequencing by synthesis using modified nucleotides (such as commercialized in TruSeq™ and HiSeq™ technology by Illumina, Inc., San Diego, CA; HeliScope™ by Helicos Biosciences Corporation, Cambridge, MA; and PacBio RS by Pacific Biosciences of California, Inc., Menlo Park, CA), sequencing by ion detection

technologies (such as Ion Torrent™ technology, Life Technologies, Carlsbad, CA); sequencing of DNA nanoballs (Complete Genomics, Inc., Mountain View, CA); nanopore-based sequencing technologies (for example, as developed by Oxford Nanopore Technologies, LTD, Oxford, UK), and like highly parallelized sequencing methods.

[0082] "SNP" or "single nucleotide polymorphism" may include a genetic variation between individuals; *e.g.*, a single nitrogenous base position in the DNA of organisms that is variable. SNPs are found across the genome, much of the genetic variation between individuals is due to variation at SNP loci, and often this genetic variation results in phenotypic variation between individuals. SNPs for use in the present disclosure and their respective alleles may be derived from any number of sources, such as public databases (U.C. Santa Cruz Human Genome Browser Gateway (genome.ucsc.edu/cgi-bin/hgGateway) or the NCBI dbSNP website (ncbi.nlm.nih gov/SNP/), or may be experimentally determined as described in U.S. Pat. No. 6,969,589; and US Pub. No. 2006/0188875 entitled "Human Genomic Polymorphisms." Although the use of SNPs is described in some of the embodiments presented herein, it will be understood that other biallelic or multi-allelic genetic markers may also be used. A biallelic genetic marker is one that has two polymorphic forms, or alleles. As mentioned above, for a biallelic genetic marker that is associated with a trait, the allele that is more abundant in the genetic composition of a case group as compared to a control group is termed the "associated allele," and the other allele may be referred to as the "unassociated allele." Thus, for each biallelic polymorphism that is associated with a given trait (*e.g.*, a disease or drug response), there is a corresponding associated allele. Other biallelic polymorphisms that may be used with the methods presented herein include, but are not limited to multinucleotide changes, insertions, deletions, and translocations.

[0083] It will be further appreciated that references to DNA herein may include genomic DNA, mitochondrial DNA, episomal DNA, and/or derivatives of DNA such as amplicons, RNA transcripts, cDNA, DNA analogs, *etc.* The polymorphic loci that are screened in an association study may be in a diploid or a haploid state and, ideally, would be from sites across the genome. Sequencing technologies are available for SNP sequencing, such as the BeadArray platform (GOLDENGATE™ assay) (Illumina, Inc., San Diego, CA) (see Fan, *et al.*, *Cold Spring Symp. Quant. Biol.*, 68:69-78 (2003)), may be employed.

[0084] In some embodiments, the term "methylation state" or "methylation status" refers to the presence or absence of 5-methylcytosine ("5-mC" or "5-mCyt") at one or a plurality of CpG dinucleotides within a DNA sequence. Methylation states at one or more particular CpG methylation sites (each having two CpG dinucleotide sequences) within a DNA sequence include "unmethylated," "fully-methylated," and "hemi-methylated." The term "hemi-methylation" or "hemimethylation" refers to the methylation state of a double stranded DNA wherein only one strand thereof is methylated. The term "hypermethylation" refers to the average methylation state corresponding to an increased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample. The term "hypomethylation" refers to the average methylation state corresponding to a decreased presence of 5-mCyt at one or a plurality of CpG dinucleotides within a DNA sequence of a test DNA sample, relative to the amount of 5-mCyt found at corresponding CpG dinucleotides within a normal control DNA sample.

[0085] "Multiplexing" or "multiplex assay" herein may refer to an assay or other analytical method in which the presence and/or amount of multiple targets, *e.g.*, multiple nucleic acid sequences, can be assayed simultaneously by using more than one markers, each of which has at least one different detection characteristic, *e.g.*, fluorescence characteristic (for example excitation wavelength, emission wavelength, emission intensity, FWHM (full width at half maximum peak height), or fluorescence lifetime) or a unique nucleic acid or protein sequence characteristic.

[0086] As used herein, "disease or disorder" refers to a pathological condition in an organism resulting from, *e.g.*, infection or genetic defect, and characterized by identifiable symptoms.

B. Overview of the polynucleotide fragment analysis by library construction and polynucleotide sequencing.

[0087] In one aspect, the target (or template) polynucleotide of the present method is a fragmented polynucleotide, for example, ranging from about 100 residues to about 1000 residues, and in some embodiments, ranging from about 150 residues to about 400 residues.

[0088] The target or template DNA can include be regular genomic DNA, chromosomal DNA, extrachromosomal DNA (such as mitochondrial DNA), or a fragment thereof. In other

embodiments, the target or template DNA is a processed DNA, for example, one that has undergone enzyme digestion, cross-linking, chemical or physical shearing, bisulfite conversion, and/or degradation.

[0089] Bisulfite conversion is a method that uses bisulfite to determine the methylation pattern of DNA. DNA methylation is a biochemical process involving the addition of a methyl group to the cytosine or adenine DNA nucleotides. DNA methylation stably alters the expression of genes in cells as cells divide and differentiate from embryonic stem cells into specific tissues. In bisulfite conversion, target nucleic acids are first treated with bisulfite reagents that specifically convert un-methylated cytosines to uracils while having no impact of methylated cytosine. One consequence of bisulfite conversion is that the double-stranded conformation of the original target is disrupted due to loss of sequence complementarity. The target sequences exist as two separate single-stranded DNAs during sample preparation and analytical or diagnostic testing. Target nucleic acid sequences frequently also exist at very low concentrations. This is an especially important consideration for circulating tumor DNA (also referred to as "cell-free tumor DNA," or "ctDNA") due to its often low concentration in circulation and the very low variant allele fraction.

[0090] In some embodiments, the nucleic acid molecule of interest disclosed herein is a cell-free DNA, such as cell-free fetal DNA (also referred to as "cfDNA") or ctDNA. cfDNA circulates in the body, such as in the blood, of a pregnant mother, and represents the fetal genome, while ctDNA circulates in the body, such as in the blood, of a cancer patient, and is generally pre-fragmented. In other embodiments, the nucleic acid molecule of interest disclosed herein is an ancient and/or damaged DNA, for example, due to storage under damaging conditions such as in formalin-fixed samples, or partially digested samples.

[0091] As cancer cells die, they release DNA into the bloodstream. This DNA, known as circulating tumor DNA (ctDNA), is highly fragmented, with an average length of approximately 150 base pairs. Once the white blood cells are removed, ctDNA generally comprises a very small fraction of the remaining plasma DNA, for example, ctDNA may constitute less than about 10% of the plasma DNA. Generally, this percentage is less than about 1%, for example, less than about 0.5% or less than about 0.01%. Additionally, the total amount of plasma DNA is generally very low, for example, at about 10 ng/mL of plasma.

[0092] The variants in the ctDNA can be interrogated using various methods, including next generation sequencing. Due to the low ratio of ctDNA to plasma DNA, it is difficult to call a variant with high confidence due to PCR and sequencing errors. Unique molecular identifiers (UMIs) are generally used to tag original molecules such that any variant seen can be compared to a consensus sequence. This is an effective manner to separate true from false positives. If the variant is matched to a consensus, it is a true positive. Otherwise, it is removed from analysis. Furthermore, it is essential that a high percentage of original molecules are turned into sequencing libraries so that the sensitivity remains high, *i.e.*, variants are not missed due to dropout. Thus, ligation efficiency is extremely important during library construction.

[0093] In one aspect, provided herein is a technique to vastly improve ligation efficiency while still targeting selected regions of the genome. In one embodiment, polynucleotides to be detected by sequencing, such as ctDNA, are first dephosphorylated to remove 5' phosphates to prevent ligation of ctDNA to itself. The ctDNA is then denatured such that all DNA is single stranded. Circligase™, a single stranded DNA ligase, is used to ligate an adapter to the 3' end of the ctDNA. In one aspect, the adapter contains 2 specific bases on the 5' end to optimize ligation efficiency, followed by a UMI. In one aspect, the 3' end of the adapter contains a carbon spacer to prevent self-ligation of the adapters. In another aspect, the ligation reaction is further optimized using a crowding agent, such as PEG 4000. In one aspect, following ligation, molecules are double-stranded using a primer that is reverse complement to the adapter. This allows efficient removal of excess unligated adapters without removed usable DNA by a standard purification.

[0094] In one aspect, the DNA is then amplified using a semi-targeted PCR. One primer is reverse complement to the adapter, while the other (*e.g.*, as one primer in a primer pool) anneals to specific, targeted regions of the genome. The specific primers were designed to minimize primer-dimer interactions and off-target annealing. In one aspect, the target-specific primers are further optimized to land in close proximity to specific variants due to the small DNA size. Following another cleanup, a PCR adds the full-length sequencing adapters and barcodes. The final library is then sequenced, for example, on an Illumina machine.

[0095] In one aspect, the semi-targeted PCR results in enrichments of > about 40,000 fold of the original molecule set despite having a relatively small target region of ~30,000 bp. In one aspect, the overall conversion rates of the present method are at least 60%, implying that at least

~3 times more of the original molecules are converted into sequenceable material when compared to standard library construction and hybridization capture. In other embodiments, the overall conversion rates are between about 60% and about 70%, between about 70% and about 80%, between about 80% and about 90%, or over 90%. In one aspect, the present method thus is able to accurately call genetic or genomic variants, such as SNVs, indels, CNVs, and fusions at extremely low mutant allele fractions, for example, as low as 0.01%. In other aspects, the allele fraction of the genetic or genomic variant is about 0.05%, about 0.1%, about 0.5%, about 1%, or about 2%.

[0096] The following sections describe certain steps of the present method in greater detail.

C. Single-stranded polynucleotide libraries and method of constructing the same.

[0097] Library construction for next generation sequencing, for example, for ctDNA, generally consists of several steps, including end repair, A-tailing, and a double stranded ligation of an adapter molecule. These ligated molecules can then be enriched 1000-2000 times at certain genomic regions using hybridization capture. Despite several improvements in library construction over the last several years, the process remains inefficient, resulting in many original molecules lost during the various steps. Double stranded ligation efficiency remains low, with ~20-30% of the molecules being properly ligated. Additionally, many molecules are lost during the purification and hybridization capture steps, so that the final conversion rate approximates 10-20%. Sensitivity remains low when interrogating low allele fraction variants found in ctDNA. This limits the accuracy when calling low allele fraction mutants, since the low efficiency will result in sensitivity loss when looking at libraries with low allele fractions.

[0098] In addition, the small size of certain polynucleotides, such as ctDNA, prevents the use of tagmentation-based library construction. For example, the polynucleotides are first tagged (*e.g.*, with biotin) to generate a targeted library, and then enriched by capturing the tags (*e.g.*, by streptavidin). This way, the library for the regions of interest can be enriched by about 1000-2000 fold. Finally, a PCR is performed to amplify and index the molecules for sequencing. However, PCR based methods prove difficult to add UMIs to original molecules and result in high error rates.

[0099] In one aspect, the compositions, kits, and methods described herein addressed the above problems. In some embodiments, the compositions, kits, and methods are useful in

sequencing nucleic acid molecules, including but not limited to construction of various libraries, various amplification reactions (such as by PCR and/or primer extension), purification of the constructed libraries, and analysis of sequencing reads.

[00100] In certain aspects, a sequencing library can be prepared, for example, from a sample containing fragmented polynucleotides, such as fragment DNA. In one aspect, the sample is obtained a naturally occurring sample, for example, directly from a subject, such as tissue fluid or body fluid, including but not limited to blood, plasma, serum, cerebrospinal fluid, synovial fluid, urine, sweat, semen, sputum, tear, mucus, or amniotic fluid. In other aspects, a sequencing library can be prepared by forming fragments of DNA (for example, by shearing the DNA), and attaching the adapters herein to the DNA fragments. In particular embodiments, the fragmented polynucleotides and the adapters are single-stranded.

[00101] The fragments (for example, the ctDNA or fragments formed by fragmenting longer DNA strands) are sometimes referred to as "inserts," as they can be "inserted" or ligated adjacent to an adapter such as a single-stranded adaptor disclosed herein. RNA molecules can also be sequenced, for example by reverse transcribing the RNA molecules to form DNA molecules, which are attached to the adapters.

[00102] In one aspect, a method comprising ligating a set of adaptors to a library of single-stranded polynucleotides is provided, and in the method, the ligation is catalyzed by a single-stranded DNA (ssDNA) ligase. As used herein, a ssDNA ligase is capable of ligating ends of ssDNA in the absence of a complementary sequence. For example, CircLigase™ ssDNA Ligase and CircLigase™ II ssDNA Ligase are both thermostable ligases that are typically used to catalyze intramolecular ligation (*i.e.*, circularization) of ssDNA templates having a 5′-phosphate and a 3′-hydroxyl group. In contrast to T4 DNA Ligase and Ampligase® DNA Ligase, which ligate DNA ends that are annealed adjacent to each other on a complementary DNA sequence, a ssDNA ligase ligates ends of ssDNA in the absence of a complementary sequence. The enzyme is therefore useful for making circular ssDNA molecules from linear ssDNA. Circular ssDNA molecules can be used as substrates for rolling-circle replication or rolling-circle transcription. In addition to its activity on ssDNA, a CircLigase enzyme also has activity in ligating a single-stranded nucleic acid having a 3′-hydroxyl ribonucleotide and a 5′-phosphorylated ribonucleotide or deoxyribonucleotide.

[00103] Either CircLigase™ ssDNA Ligase or CircLigase™ II ssDNA Ligase can be used in the present disclosure. The two enzymes are different in that CircLigase I is far less adenylated than CircLigase II and requires ATP for best activity. CircLigase I recircularizes ssDNA in the presence of ATP. CircLigase II is nearly 100% adenylated, therefore it is not necessary to add ATP to the reaction buffer. CircLigase II works as a stoichiometric reaction, where the enzyme bonds the 5′-end of an oligo that is adenylated in the enzyme active site, and then ligates the oligo and stops. Since the reaction doesn′t contain ATP, CircLigase II works in a 1:1 enzyme:oligo configuration. Once the circularization is complete, the circular ssDNA is released from the active site and the reaction stops. Other suitable ssDNA ligase can also be used. For example, a thermostable 5' App DNA/RNA ligase, *e.g.*, New England Biosciences, M0319S, or T4 DNA ligase, *e.g.*, New England Biosciences, M0202S, or a T4 RNA ligase, *e.g.*, T4 RNA ligase I, *e.g.*, New England Biosciences, M0204S, T4 RNA ligase 2, *e.g.*, New England Biosciences, M0239S, T4 RNA ligase 2 truncated, *e.g.*, New England Biosciences, M0242S, T4 RNA ligase 2 truncated KQ, *e.g.*, M0373S, or T4 RNA ligase 2 truncated K227Q, *e.g.*, New England Biosciences, M0351S, can be used.

[00104] In one aspect, each single-stranded polynucleotide is blocked at the 5' end to prevent ligation at the 5' end, each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide to which the adaptor is ligated, each adaptor is blocked at the 3' end to prevent ligation at the 3' end, and the 5' end of the adaptor is ligated to the 3' end of the single-stranded polynucleotide by the ssDNA ligase to form a linear ligation product, thereby obtaining a library of linear, single-stranded ligation products. Template-independent circularization of single-stranded DNA is described in WO2010/094040 A1, the disclosure of which is incorporated herein in its entirety. WO2010/094040 A1, however, only discloses intramolecular ligation (*e.g.*, circularization) of single-stranded polynucleotides.

[00105] Thus, the present method uses a ssDNA ligase, such as CircLigase or CircLigase II, in an unconventional manner. Instead of circularization, the present ligation method aims to generate a linear ligation product between the single-stranded target polynucleotide and an adaptor molecule. In one aspect, the present disclosure uses a ssDNA ligase to carry out intramolecular ligate, *e.g.*, for ligating an adaptor to single-stranded polynucleotides. In order to do, in one aspect, the single-stranded polynucleotide is blocked at the 5' end to prevent circularization. This way, intramolecular ligation of the 3' end of an ssDNA to its own 5' end,

as well as intermolecular ligation of the 3' end of one ssDNA to the 5' end of another ssDNA within the same library, is prevented. Thus, in one aspect, both circularization of the single-stranded polynucleotide and formation of linear concatemers (containing the single-stranded polynucleotides and/or the adaptors) are prevented during the ligation reaction. As shown in **FIG. 1**, the blocking of each single-stranded polynucleotide can comprise dephosphorylation at its 5' end to prevent ligation at that end.

[00106] In another aspect, each adaptor is blocked at the 3' end to prevent ligation at the 3' end. This way, intramolecular ligation of the 3' end of an adaptor to its own 5' end, as well as intermolecular ligation of the 3' end of one adaptor molecule to the 5' end of another adaptor molecule, is prevented. The blocking of each adaptor can comprise a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol (TEG), and/or hexaethylene glycol, to prevent ligation at its 3' end. Thus, in one aspect, both circularization of the single-stranded adaptor and formation of linear concatemers (containing the single-stranded polynucleotides and/or the adaptors) are prevented during the ligation reaction.

[00107] The adaptor may comprise one or more copies of one or more spacers, in any suitable combination. For example, Gansauge and Meyer disclosed an adaptor that comprises ten copies of a C3Spacer and a biotinylated TEG spacer. Gansauge and Meyer (2013), "Single-stranded DNA library preparation for the sequencing of ancient or damaged DNA," *Nature Protocols*, 8(4): 737-48, which is incorporated herein by reference in its entirety. This reference, however, requires capturing the ligated ssDNA, via biotin-streptavidin interaction, immediately after ligation. This step may cause a significant loss of the ssDNA molecules in the library. The reference then converts the captured ssDNA to dsDNA while the ssDNA remains captured on a bead.

[00108] As shown in **FIG. 1**, the present disclosure does not require capturing the ligated ssDNA immediately after ligation. Instead, the ligated ssDNA remains in the ligation reaction volume when it is converted into dsDNA.

[00109] In one aspect, the ligation efficiency of the ssDNA in the library is high, for example, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 99% of the single-stranded polynucleotides in the sample are ligated to an adaptor. In particular embodiments, the ligation efficiency is about 80%. With this vastly improved ligation

efficiency, the presently claimed method is still capable of targeting selected regions of the genome, as explained below.

[00110] In one aspect, the adaptor has the following structure: /5'Phos/N_1N_2. . .N_i-UMI-M_1M_2. . .M_j-Blocker, wherein "5'Phos" represents a 5' phosphate group, "N_1N_2. . .N_i" represents the sequence 5' to the UMI sequence, "M_1M_2. . .M_j" represents the sequence 3' to the UMI sequence, and "Blocker" indicates that the 3' end of the adaptor is blocked to prevent ligation thereto. Both *i* and *j* are integers, wherein *i* can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or greater than 30; and *j* can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, or greater than 50. In specific embodiments, *i* can be 2. In some embodiments, the dinucleotide sequence N_1N_2 at the 5' end of N_1N_2. . .N_i can be GA (5' to 3'), GG (5' to 3'), AA (5' to 3'), or AG (5' to 3'), in order to enhance the ligation efficiency.

[00111] In one aspect, a portion or all of the M_1M_2. . .M_j sequence is used in later steps for designing a reverse-complement sequence that is used as a primer to convert the ligated single-stranded polynucleotide into a double-stranded polynucleotide, and/or for the semi-targeted PCR to amplify a selected target sequence (the other primer of the primer pair being the target-specific primer). In one aspect, the M_1M_2. . .M_j sequence comprises AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 1) or a portion thereof that comprises between about 18 and 22 nucleotide residues.

[00112] In another aspect, the "Blocker" comprises a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol (TEG), and/or hexaethylene glycol, in one or more copies of one or more blocker groups in any suitable combination and order in the 5' to 3' direction.

[00113] In one aspect, use of the UMI facilitates the determination, selection, and/or isolation of error-free sequencing reads of a target sequence, and the sequencing reads can be selected with high accuracy and high throughput. Such validated, error-free sequencing reads are useful in any technique that requires sequence fidelity, including the construction of larger molecules of known sequence, polymorphism and/or mutation screening, massively parallel sequencing, and quantification methods to preclude bias in the methodologies.

[00114] In one aspect, the Unique Molecular Identifier is associated with and uniquely identifies a ligated construct comprising a single-stranded target polynucleotide and an adaptor.

In other words, two single-stranded target polynucleotides having the same sequence may be ligated to two different adaptors which differ from each other at their UMI sequences; the resultant ligation products are different, and each ligation product (rather than the target polynucleotides having the same sequence) is uniquely identified by the UMI. In another aspect, when the single-stranded ligation products are converted into double-stranded polynucleotides and amplified, amplification errors may be introduced during repeated copying even though very high fidelity polymerases are available. As a result, even a low error rate can have a significant impact, particularly in the construction of large libraries. Although massively parallel sequencing has advantages in cost and throughput, the accuracy of the reads can be comprised by the limitations of the amplification and/or detection technologies.

[00115] By using the UMI, the present method is capable of identifying error-free amplification products and/or sequencing reads, and excluding those with technical errors from analysis. The amplification products and/or sequencing reads having the same UMI can be confirmed as related (identical by descent), and thus sequence differences between molecules with the same UMI can be identified as technical errors rather than real differences in the sequence (*e.g.*, sequence differences between a wild-type sequence and a cancer-related mutant sequence). In other words, since each single-stranded ligation product is unique identifiable by its UMI, all of its descendants (due to amplification and/or sequencing) should have the same target sequence if no technical error is introduced. If, however, an error such as a single-nucleotide insertion is introduced into the target sequence during amplification and/or sequencing, some amplification products and/or sequencing reads identical by descent (*e.g.*, sharing the same UMI) will have the insertion while the others will not. The exact ratio between the products having the insertion and those that do not have the insert will vary, depending on when the error occurs during the amplification and/or sequencing process. In general, when very high fidelity polymerases are used, the products without errors will be in the majority. In another aspect, because amplification products and/or sequencing reads that are identical by descent can be identified, a consensus sequence can be determined using data from multiple molecules, thereby achieving a high accuracy for high throughput sequencing.

[00116] In one aspect, the UMI is a degenerate nucleic acid sequence, and the number of nucleotides in the UMI is designed such that the number of potential and actual sequences represented by the UMI sequences is greater than the total number of target single-stranded

target polynucleotide in the initial library. In one aspect, UMI sequence diversity (or "uniqueness" with regard to each single UMI sequence) can be provided by using a degenerate collection of sequences randomly generated by synthesizing with a mixture of all four bases at each position. Alternatively, a diverse but pre-defined set of sequences can be synthesized and ligated to the initial single-stranded polynucleotide library. The diversity of the UMI set needs to be sufficient so that molecules that are not related by descent won't be mistaken as such. In one aspect, a "unique" molecular identifier need not be absolutely unique, and may be used on different target single-stranded polynucleotides provided it is clear that they are different and not mistaken for a molecule that is identical by descent. The large number of UMI sequences that can be generated from the random assembly of nucleotides provides a high probability that each individual ligation product can be uniquely identified. For example, if the UMI comprises a 12-mer synthesized with a mixture of A, C, G and T at each position, there are 4^{12} possible sequences. If the UMI comprises a 20-mer synthesized with a mixture of A, C, G and T at each position, there are 4^{20} (about 10^{12}) possible sequences. The use of such random identifiers allows a large library with single-stranded target polynucleotides that can be individually distinguished from each other.

[00117] In particular aspects, the UMI is a 5-mer, 6-mer, 7-mer, 8-mer, 9-mer, 10-mer, 11-mer, 12-mer, 13-mer, 14-mer, 15-mer, 16-mer, 17-mer, 18-mer, 19-mer, 20-mer, 21-mer, 22-mer, 23-mer, 24-mer, 25-mer, or even longer degenerate sequence. In one aspect, the adaptor has the following structure: /5'Phos/ GANNNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG/3SpC3/, wherein "NNNNNNNNNNNN" represents a 12-mer UMI sequence, and "3SpC3" represents a 3' carbon spacer. The sequence of GANNNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG is SEQ ID NO: 2.

[00118] The concentration of DNA can be artificially increased by adding condensing agents such as cobalt hexamine and biogenic polyamines such as spermidine, or by using crowding agents such as polyethylene glycol (PEG) which also increase the effective concentration of enzymes. In one aspect, additives such as cobalt hexamine can produce exclusively intermolecular reaction, resulting in linear ligation products rather than circular products. Thus, in case the 5' ends of the single-stranded target polynucleotides and the 3' ends of the single-

stranded adaptor may not be completely blocked to prevent ligation, additives such as cobalt hexamine may be used to enhance intermolecular reaction and further prevent circularization of the single-stranded target polynucleotide and/or the adaptor.

[00119] In some embodiments, more than one configurations of the adaptor can be used in the same ligation reaction. For example, two configurations of the adaptor may be used:

Configuration No. 1: /5`Phos/N_1N_2. . .N_i-UMI_1-M_1M_2. . .M_j-$Blocker_1$, and

Configuration No. 2: /5`Phos/P_1P_2. . .P_k-UMI_2-Q_1Q_2. . .Q_l-$Blocker_2$.

[00120] N_1N_2. . .N_i and P_1P_2. . .P_k can be the same or different, UMI_1 and UMI_2 can be the same or different, M_1M_2. . .M_j and Q_1Q_2. . .Q_l, can be the same or different, and $Blocker_1$ and $Blocker_2$ can be the same or different. In one embodiment, UMI_1 is different from UMI_2 (for example, UMI_1 is a 12-mer degenerate sequence while UMI_2 is a 13-mer degenerate sequence), while the other features of the adaptors are the same. In another embodiment, N_1N_2. . .N_i is different from P_1P_2. . .P_k (for example, one is AG while the other is GA), while the other features of the adaptors are the same. In yet another embodiment, M_1M_2. . .M_j is different from Q_1Q_2. . .Q_l, while the other features of the adaptors are the same. In still another embodiment, $Blocker_1$ and $Blocker_2$ are different, while the other features of the adaptors are the same.

[00121] After the ligation reaction, the single-stranded ligation products, without any need for purification (*e.g.*, separation of the ligation products from the excess, unligated adaptor molecules), can be immediately subject to conversion into double-stranded ligation products. In addition, neither the single-stranded target polynucleotide nor the adaptor needs to be captured on a solid support (*e.g.*, by biotin-streptavidin mediated binding to a bead) in order for the subsequent conversion of the ligation product into a double-stranded polynucleotide and/or amplification step. Thus, the present method avoids and/or reduces loss of the already small allele fraction of the mutant in a DNA sample, such as ctDNA, due to the purification or isolation of the single-stranded ligation products. Instead, in one aspect, the single-stranded ligation products remain in the solution which is directed subject to primer extension.

D. Conversion of single-stranded polynucleotide library to double-stranded polynucleotide library.

[00122] In one aspect as shown in **FIG. 1**, following construction of the library containing the single-stranded ligation products, the method can further comprise converting the library of linear, single-stranded ligation products into a library of linear, double-stranded ligation

products. In one aspect, the conversion uses a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor.

[00123] For an adaptor having the following structure: /5'Phos/N_1N_2. . .N_i-UMI-M_1M_2. . .M_j-Blocker, the primer can comprise a sequence that is reverse-complement and/or hybridizable to M_1M_2. . .M_j. In this example, when the primer hybridizes to the ligated product having the structure ssDNA-N_1N_2. . .N_i-UMI-M_1M_2. . .M_j-Blocker, the primer extension reaction can convert the ssDNA-N_1N_2. . .N_i-UMI sequence (and optionally, all or a portion of the M_1M_2. . .M_j sequence) into double-stranded polynucleotides. In one specific example, a reverse-complement primer comprises the sequence set forth in SEQ ID NO: 3: CACTCTTTCCCTACACGACGC (5' to 3').

[00124] In some embodiments, the primer may not be a perfect reverse-complement of M_1M_2. . .M_j or a portion therefore; nonetheless, the primer is hybridizable to M_1M_2. . .M_j (and thus the ssDNA ligated to the adaptor) under stringent conditions.

[00125] In any of the preceding embodiments, the method can further comprise amplifying and/or purifying the library of linear, double-stranded ligation products. In one aspect, the double-stranded ligation products are purified and size selected to remove unbound adaptor molecules and/or unbound primers, and/or complexes formed between an adaptor and its reverse-complement primer. Any suitable methods can be used to remove these fragments which are generally shorter than the desired double-stranded ligation products. For example, using PCR purification column from Qiagen could help to eliminate the smaller fragments from the samples and running the column-purified samples on 2% certified low range ultra agarose gel can help to select the desired fragment size. The beads-based DNA purification including AMPure method is also helpful to remove the smaller fragments. In some embodiments, the desired double-stranded ligation products size is from about 100bps to about 600 bps, such as from about 100bps to about 400 bps, from about 150bps to about 200 bps, from about 200bps to about 250 bps, and from about 250bps to about 300 bps. In one embodiment, dsDNA (>150 bps and <400 bps) is purified and collected, for example, by eluting beads suspended in a Tri-EDTA buffer.

[00126] In one aspect, the purification is bead-based. In another aspect, the purification is based on size selection, for example, the purification step selectively purifies polynucleotides between about 50 nucleotides and about 1000 nucleotides in lengths, for example, adaptors of

about 40 nucleotides in length (and primer dimers and/or primer-adaptor duplexes of about 40bp) are removed. In one aspect, the purification is column-based, for example, by using a dsDNA or ssDNA purification column, such as those from Zymo or Qiagen.

[00127] In another aspect, the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead).

[00128] In any of the preceding embodiments, the method herein can further comprise amplifying the library of linear, double-stranded ligation products, *e.g.*, by a polymerase chain reaction (PCR), to obtain an amplified library of linear, double-stranded ligation products comprising sequence information of a target sequence. This amplification can be an unbiased amplification, for example, by ligating a universal adaptor pair to the ends of the double-stranded ligation products, and amplifying all the tagged double-stranded ligation products with a universal primer pair. In other embodiments, a semi-targeted amplification is conducted in lieu of or in addition to the unbiased amplification. The semi-targeted amplification can be performed before or after the unbiased amplification.

E. Semi-targeted amplification of double-stranded polynucleotide library.

[00129] In one aspect, as shown in **FIG. 1**, a semi-targeted amplification of the double-stranded ligation product library comprises using a primer comprising a sequence that is reverse-complement and/or hybridizable to the adaptor, and a primer hybridizable to a target sequence (*e.g.*, an EGFR gene sequence) or primers hybridizable to the same target sequence or multiple target sequences.

[00130] For an adaptor having the following structure: /5'Phos/N_1N_2. . .N_i-UMI-M_1M_2. . .M_j-Blocker, the primer can comprise a sequence that is reverse-complement and/or hybridizable to M_1M_2. . .M_j. This way, when the primer hybridizes to one strand of the dsDNA and the target-specific primer hybridizes to the other strand of the dsDNA, the PCR product will contain a target sequence as well as the N_1N_2. . .N_i-UMI sequence (and optionally, all or a portion of the M_1M_2. . .M_j sequence). In one specific example, a reverse-complement primer comprises the sequence set forth in SEQ ID NO: 3: CACTCTTTCCCTACACGACGC (5' to 3').

[00131] In one aspect, a plurality of target-specific primers can be used, each comprising a sequence specific for the same or a different target sequence. In other words, the primers can

have the same or different target sequences. In some embodiments, the pool of target-specific primers comprises about 5, about 10, about 25, about 50, about 100, about 150, about 200, about 250, about 300, about 400, about 500, about 600, about 700, about 800, about 900, about 1000, or more than about 1000 different primers, such as about 10^4, about 10^5, about 10^6, or more primers. In other embodiments, the pool comprises between about 20 and about 60, between about 60 and about 100, between about 100 and about 140, between about 140 and about 180, between about 180 and about 220, between about 220 and about 260, between about 260 and about 300, between about 300 and about 350, or between about 350 and about 400 different primers. In one aspect, the pool of target-specific primers are used together with one common reverse-complement primer, wherein the common reverse-complement primer forms a primer pair with each individual target-specific primer in the pool to amplify the target sequence in between the primers in a semi-targeted fashion. Thus, in this aspect, the semi-targeted amplification is not a whole genome amplification.

[00132] Since ctDNA fragments randomly, in one aspect, the primer position of the target-specific primer may be important. For example, if the primer landing spans a break point, it may result in lower conversion rates. A larger target-specific primer pool and/or using multiple partially overlapping primers for the same target sequence may solve the problem.

[00133] In one aspect, the sequence information of the target sequence can comprise a mutation, a single nucleotide polymorphism (SNP), a copy number variation (CNV), or an epigenetic change. In one aspect, the mutation comprises a point mutation, an insertion, a deletion, an inversion, a truncation, a fusion, an amplification, or any combination thereof.

[00134] In some embodiments, the amplified library of linear, double-stranded ligation products can be a library other than a whole genome library, for example, a semi-targeted genome library.

[00135] In some embodiments, the method can further comprise purifying the amplified library of linear, double-stranded ligation products. Any suitable methods can be used to remove smaller fragments including primer dimers. For example, using PCR purification column from Qiagen could help to eliminate the smaller fragments from the samples and running the column-purified samples on 2% certified low range ultra agarose gel can help to select the desired fragment size. The beads-based DNA purification including AMPure method is also helpful to remove the smaller fragments. In some embodiments, the amplification

product size is from about 100bps to about 600 bps, such as from about 100bps to about 400 bps, from about 150bps to about 200 bps, from about 200bps to about 250 bps, and from about 250bps to about 300 bps. In one embodiment, dsDNA (>150 bps and <400 bps) is purified and collected, for example, by eluting beads suspended in a Tri-EDTA buffer.

[00136] In one aspect, the purification is bead-based. In another aspect, the purification is based on size selection, for example, the purification step selectively purifies polynucleotides greater about 150 nucleotides in lengths. In another aspect, the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead). In one aspect, the purification is column-based, for example, by using a dsDNA or ssDNA purification column, such as those from Zymo or Qiagen.

F. Construction of sequence library and analysis of sequencing reads.

[00137] In one aspect, the method further comprises sequencing the purified amplified library of linear, double-stranded ligation products. In one aspect, the sequencing step comprises attaching a sequencing adapter and/or a sample-specific barcode to each linear, double-stranded ligation product. In one particular aspect, the attaching step is performed using a polymerase chain reaction (PCR).

[00138] **FIG. 2** shows an exemplary configuration of a construct comprising a target molecule for sequencing. For Illumina sequencing, on each end, these constructs have flow cell binding sites, P5 and P7, which allow the library fragment to attach to the flow cell surface. The P5 and P7 regions of single-stranded library fragments anneal to their complementary oligos on the flowcell surface. The flow cell oligos act as primers and a strand complementary to the library fragment is synthesized. Then, the original strand is washed away, leaving behind fragment copies that are covalently bonded to the flowcell surface in a mixture of orientations. Copies of each fragment are then generated by bridge amplification, creating clusters. Then, the P5 region is cleaved, resulting in clusters containing only fragments which are attached by the P7 region. This ensures that all copies are sequenced in the same direction. The sequencing primer anneals to the P5 end of the fragment, and begins the sequencing by synthesis process. Index reads are performed when a sample is barcoded. When Read 1 is finished, everything from Read 1 is removed and an index primer is added, which anneals at the P7 end of the

fragment and sequences the barcode. Then, everything is stripped from the template, which forms clusters by bridge amplification as in Read 1. This leaves behind fragment copies that are covalently bonded to the flowcell surface in a mixture of orientations. This time, P7 is cut instead of P5, resulting in clusters containing only fragments which are attached by the P5 region. This ensures that all copies are sequenced in the same direction (opposite Read 1). The sequencing primer anneals to the P7 region and sequences the other end of the template.

[00139] Next-generation sequencing platforms, such as MiSeq (Illumina Inc., San Diego, CA), can be used for highly multiplexed assay readout. A variety of statistical tools, such as the Proportion test, multiple comparison corrections based on False Discovery Rates (*see* Benjamini and Hochberg, 1995, *Journal of the Royal Statistical Society Series B* (Methodological) 57, 289-300), and Bonferroni corrections for multiple testing, can be used to analyze assay results. In addition, approaches developed for the analysis of differential expression from RNA-Seq data can be used to reduce variance for each target sequence and increase overall power in the analysis. *See* Smyth, 2004, Stat. Appl. Genet. Mol. Biol. 3, Article 3.

[00140] Overall, in some embodiments, the conversion rate of the present method is at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90%, or at least about 95%. In one aspect, the conversion rate is the percentage of targeted single-stranded polynucleotides in the initial library that give rise to sequencing reads.

[00141] In any of the preceding embodiments, the method can be used for the diagnosis and/or prognosis of a disease or condition in a subject, predicting the responsiveness of a subject to a treatment, identifying a pharmacogenetics marker for the disease/condition or treatment, and/or screening a population for a genetic information. In one aspect, the disease or condition is a cancer or neoplasia, and the treatment is a cancer or neoplasia treatment.

[00142] Mutant DNA molecules offer unique advantages over cancer-associated biomarkers because they are so specific. Though mutations occur in individual normal cells at a low rate (about 10^{-9} to 10^{-10} mutations/bp/generation), such mutations represent such a tiny fraction of the total normal DNA that they are orders of magnitude below the detection limit of certain art methods. Several studies have shown that mutant DNA can be detected in stool, urine, and blood of CRC patients (Osborn and Ahlquist, Stool screening for colorectal cancer: molecular approaches, *Gastroenterology* 2005;128:192-206).

[00143] Based on the sequencing results herein, detection of circulating tumor DNA in the patient can be made, and diagnosis of cancer and predictions regarding tumor recurrence can be made. Based on the predictions, treatment and surveillance decisions can be made. For example, circulating tumor DNA which indicates a future recurrence, can lead to additional or more aggressive therapies as well as additional or more sophisticated imaging and monitoring. Circulating DNA refers to DNA that is ectopic to a tumor.

[00144] Samples which can be monitored for ctDNA include blood and stool. Blood samples may be for example a fraction of blood, such as serum or plasma. Similarly stool can be fractionated to purify DNA from other components. Tumor samples are used to identify a somatically mutated gene in the tumor that can be used as a marker of tumor in other locations in the body. Thus, as an example, a particular somatic mutation in a tumor can be identified by any standard means known in the art. Typical means include direct sequencing of tumor DNA, using allele-specific probes, allele-specific amplification, primer extension, *etc.* Once the somatic mutation is identified, it can be used in other compartments of the body to distinguish tumor derived DNA from DNA derived from other cells of the body. Somatic mutations are confirmed by determining that they do not occur in normal tissues of the body of the same patient. Types of tumors which can be diagnosed and/or monitored in this fashion are virtually unlimited. Any tumor which sheds cells and/or DNA into the blood or stool or other bodily fluid can be used. Such tumors include, in addition to colorectal tumors, tumors of the breast, lung, kidney, liver, pancreas, stomach, brain, head and neck, lymphatics, ovaries, uterus, bone, blood, *etc.*

[00145] In one aspect, the method disclosed herein can be used to construct a library for use in sequencing and/or in determining an epigenetic status/state of one or more regions of the target sequence. DNA methylation was first the discovered epigenetic mark. Epigenetics is the study of changes in gene expression or cellular phenotype caused by mechanisms other than changes in the underlying DNA sequence. Methylation predominately involves the addition of a methyl group to the carbon-5 position of cytosine residues of the dinucleotide CpG and is associated with repression or inhibition of transcriptional activity.

[00146] Bisulfite conversion is the use of bisulfite reagents to treat DNA to determine its pattern of methylation. The treatment of DNA with bisulfite converts cytosine residues to uracil but leaves 5-methylcytosine residues unaffected. Thus, bisulfite treatment introduces specific

changes in the DNA sequence that depend on the methylation status of the individual cytosine residues. Various analyses can be performed on the altered sequence to retrieve this information, for example, in order to differentiate between single nucleotide polymorphisms (SNP) resulting from the bisulfite conversion. U.S. Patent No. 7,620,386, U.S. Patent No. 9,365,902, and U.S. Patent Application Publication 2006/0134643, all of which are incorporated herein by reference, exemplify methods known to one of ordinary skill in the art with regard to detecting sequences altered due to bisulfite conversion. Bisulfite conversion can be conducted using any suitable techniques, procedures or reagents. In some embodiments, bisulfite conversion can be conducted using any of the following kits and procedures provided in the kit: EpiMark Bisulfite Conversion Kit, New England Biosciences, E3318S; EZ DNA Methylation Kit, Zymo Research, D5001; MethylCode Bisulfite Conversion Kit, Thermo Fisher Scientific, MECOV50; EZ DNA Methylation Gold Kit, Zymo Research, D5005; EZ DNA Methylation Direct Kit, Zymo Research, D5020; EZ DNA Methylation Lightning Kit, Zymo Research, D5030T; EpiJET Bisulfite Conversion Kit, Thermo Fisher Scientific, K1461; or EpiTect Bisulfite Kit, Qiagen, 59104.

[00147] As discussed above, one consequence of bisulfite conversion is that the double-stranded conformation of the original target is disrupted due to loss of sequence complementarity. While this may cause problem for traditional methods for constructing double-stranded libraries, in one aspect the present method is uniquely suited to construct single-stranded libraries from bisulfite conversion sample for sequencing analysis.

[00148] In another aspect, the present method can be used in combination with a method for determining a methylation state/status, for example, as described in U.S. Provisional Application No. 62/487,422, entitled "Compositions and Methods for Detection of Genomic Variance and DNA Methylation Status," filed April 19, 2017 (Attorney Docket No. 737993000100), which is incorporated herein by reference in its entirety for all purposes. In one embodiment, a sample is contacted with a methylation-sensitive restriction enzyme (MSRE) before the dephosphorylation and/or the denaturing step, and methylation profiles are then be analyzed by constructing a single-stranded library by ligation as disclosed herein.

G. Kit for library construction and/or sequencing.

[00149] Disclosed in another aspect herein is a kit for constructing a library of ligation products. In one embodiment, the kit comprises a single-stranded DNA (ssDNA) ligase. In another aspect, the kit comprises a plurality of adaptors. In particular aspects, each adaptor is blocked to prevent ligation at the 3' end while the 5' end of the adaptor is available for ligation to a single-stranded polynucleotide to form a linear, single-stranded ligation product. In further particular aspects, each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide.

[00150] In one aspect, the kit for constructing a library of ligation products can comprise a ssDNA ligase and a plurality of adaptors, and each adaptor is blocked to prevent ligation at the 3' end while the 5' end of the adaptor is available for ligation to a single-stranded polynucleotide to form a linear, single-stranded ligation product, and each adaptor comprises a UMI sequence that earmarks the single-stranded polynucleotide.

[00151] In another aspect, the kit can further comprise a denaturing reagent for denaturing a double-stranded polynucleotide from a sample to obtain the single-stranded polynucleotide.

[00152] In still another aspect, the kit can comprise a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), or an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1. In any of the preceding embodiments, the kit can comprise an RNA ligase, such as a T4 RNA ligase, *e.g.*, T4 RNA ligase 2, T4 RNA ligase 2 truncated, T4 RNA ligase 2 truncated KQ, or T4 RNA ligase 2 truncated K227Q. The present kit can also comprise other suitable ssDNA ligase, *e.g.*, T4 RNA ligase I, thermostable 5' App DNA/RNA ligase, T4 RNA ligase 2, truncated T4 RNA ligase 2, *e.g.*, T4 RNA ligase 2 Truncated, T4 RNA ligase2 Truncated K227Q, T4 RNA ligase2 Truncated KQ, or T4 DNA ligase.

[00153] In one aspect, the kit can further comprise a crowding agent for the ligation reaction. In one aspect, the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000 or PEG 6000, Dextran, and/or Ficoll.

[00154] In another aspect, the kit can further comprise a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor, for converting the single-stranded polynucleotide to a double-stranded polynucleotide.

[00155] In one aspect, the kit can further comprise a reagent for removing primer dimer and/or primer-adaptor duplex.

[00156] In another aspect, the kit can further comprise a primer comprising a sequence specific for a target sequence (*e.g.*, an EGFR gene sequence), for obtaining an amplified linear, double-stranded ligation product comprising sequence information of the target sequence. In a further aspect, the kit can further comprise a sequencing adapter and/or a sample-specific barcode, for sequencing the amplified linear, double-stranded ligation product.

[00157] Diagnostic kits based on the kit components described above are also provided, and they can be used to diagnose a disease or condition in a subject, for example, cancer. In another aspect, the kit can be used to predict individual's response to a drug, therapy, treatment, or a combination thereof. Such test kits can include devices and instructions that a subject can use to obtain a sample, *e.g.*, of ctDNA, without the aid of a health care provider.

[00158] For use in the applications described or suggested above, kits or articles of manufacture are also provided. Such kits may comprise at least one reagent specific for genotyping a marker for a disease or condition, and may further include instructions for carrying out a method described herein.

[00159] In some embodiments, provided herein are compositions and kits comprising primers and primer pairs, which allow the specific amplification of the polynucleotides or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules or to any part thereof for the purpose of detection, either qualitatively or quantitatively. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Such probes and primers can be used to detect the presence of polynucleotides in a sample and as a means for detecting cell expressing proteins encoded by the polynucleotides. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided herein and used effectively to amplify, clone and/or determine the presence and/or levels of polynucleotides, such as genomic DNAs, mtDNAs, and fragments thereof.

[00160] In some embodiments, the kit may additionally comprise reagents for detecting presence of polypeptides. Such reagents may be antibodies or other binding molecules that specifically bind to a polypeptide. In some embodiments, such antibodies or binding molecules

may be capable of distinguishing a structural variation to the polypeptide as a result of polymorphism, and thus may be used for genotyping. The antibodies or binding molecules may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme. Other reagents for performing binding assays, such as ELISA, may be included in the kit.

[00161] In some embodiments, the kits comprise reagents for genotyping at least two, at least three, at least five, at least ten, or more markers. The markers may be a polynucleotide marker (such as a cancer-associated mutation or SNP) or a polypeptide marker (such as overexpression or a post-translational modification, including hyper- or hypo-phosphorylation, of a protein) or any combination thereof. In some embodiments, the kits may further comprise a surface or substrate (such as a microarray) for capture probes for detecting of amplified nucleic acids.

[00162] The kits may further comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a probe that is or can be detectably labeled. Such probe may be a polynucleotide specific for a biomarker. The kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

[00163] The kit typically comprises the container(s) described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use. A label may be present on the container to indicate that the composition is used for a specific therapy or non-therapeutic application, and may also indicate directions for either *in vivo* or *in vitro* use, such as those described above.

[00164] The kit can further comprise a set of instructions and materials for preparing a tissue or cell or body fluid sample and preparing nucleic acids (such as ctDNA) from the sample.

H. Further exemplary embodiments

[00165] In any of the preceding embodiments, the ssDNA ligase can be a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and

CircLigase II™), or an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1. In other aspects, the ssDNA ligase is an RNA ligase, such as a T4 RNA ligase, *e.g.*, T4 RNA ligase I, *e.g.*, New England Biosciences, M0204S, T4 RNA ligase 2, *e.g.*, New England Biosciences, M0239S, T4 RNA ligase 2 truncated, *e.g.*, New England Biosciences, M0242S, T4 RNA ligase 2 truncated KQ, *e.g.*, M0373S, or T4 RNA ligase 2 truncated K227Q, *e.g.*, New England Biosciences, M0351S. In any of the preceding embodiments, the ssDNA ligase can also be a thermostable 5' App DNA/RNA ligase, *e.g.*, New England Biosciences, M0319S, or T4 DNA ligase, *e.g.*, New England Biosciences, M0202S.

[00166] In some embodiments, the present methods comprise ligating a set of adaptors to a library of single-stranded polynucleotides using a single-stranded DNA (ssDNA) ligase. Any suitable ssDNA ligase, including the ones disclosed herein, can be used. The adaptors can be used at any suitable level or concentration, *e.g.*, from about 1 μM to about 100 μM such as about 1 μM, 10 μM, 20 μM, 30 μM, 40 μM, 50 μM, 60 μM, 70 μM, 80 μM, 90 μM, or 100 μM, or any subrange thereof. The adapter can comprise or begin with any suitable sequences or bases. For example, the adapter sequence can begin with all 2 bp combinations of bases.

[00167] In some embodiments, the ligation reaction can be conducted in the presence of a crowding agent. In one aspect, the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000, PEG 6000, or PEG 8000, Dextran, and/or Ficoll. The crowding agent, *e.g.*, PEG, can be used at any suitable level or concentration. For example, the crowding agent, *e.g.*, PEG, can be used at a level or concentration from about 0% (w/v) to about 25% (w/v), *e.g.*, at about 0% (w/v), 1% (w/v), 2% (w/v), 3% (w/v), 4%(w/v), 5% (w/v), 6% (w/v), 7%(w/v), 8% (w/v), 9%(w/v), 10% (w/v), 11%(w/v), 12% (w/v), 13%(w/v), 14% (w/v), 15% (w/v), 16% (w/v), 17% (w/v), 18% (w/v), 19% (w/v), 20% (w/v), 21% (w/v), 22% (w/v), 23% (w/v), 24% (w/v), or 25% (w/v), or any subrange thereof.

[00168] In some embodiments, the ligation reaction can be conducted for any suitable length of time. For example, the ligation reaction can be conducted for a time from about 2 to about 16 hours, %, *e.g.*, for about 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, 7 hours, 8 hours, 9 hours, 10 hours, 11 hours, 12 hours, 13 hours, 14 hours, 15 hours, or 16 hours, or any subrange thereof.

[00169] In some embodiments, the ssDNA ligase in the ligation reaction can be used in any suitable volume. For example, the ssDNA ligase in the ligation reaction can be used at a volume

from about 0.5 µl to about 2 µl, %, *e.g.*, at about 0.5 µl, 0.6 µl, 0.7 µl, 0.8 µl, 0.9 µl 1 µl, 1.1 µl, 1.2 µl, 1.3 µl, 1.4 µl, 1.5 µl, 1.6 µl, 1.7 µl, 1.8 µl, 1.9 µl, or 2 µl, or any subrange thereof.

[00170] In some embodiments, the ligation reaction can be conducted in the presence of a ligation enhancer, *e.g.*, betaine. The ligation enhancer, *e.g.*, betaine, can be used at any suitable volume, *e.g.*, from about 0 µl to about 1 µl, *e.g.*, at about 0 µl, 0.1 µl, 0.2 µl, 0.3 µl, 0.4 µl, 0.5 µl, 0.6 µl, 0.7 µl, 0.8 µl, 0.9 µl, 1 µl, or any subrange thereof.

[00171] In some embodiments, the ligation reaction can be conducted using a T4 RNA ligase I, *e.g.*, the T4 RNA ligase I from New England Biosciences, M0204S, in the following exemplary reaction mix (20 µl): 1 X Reaction Buffer (50 mM Tris-HCl, pH 7.5, 10 mM MgCl2, 1 mM DTT), 25% (wt/vol) PEG 8000, 1 mM hexamine cobalt chloride (optional), 1 µl (10 units) T4 RNA Ligase, and 1 mM ATP. The reaction can be incubated at 25°C for 16 hours. The reaction can be stopped by adding 40 µl of 10 mM Tris-HCl pH 8.0, 2.5 mM EDTA.

[00172] In some embodiments, the ligation reaction can be conducted using a Thermostable 5' App DNA/RNA ligase, *e.g.*, the Thermostable 5' App DNA/RNA ligase from New England Biosciences, M0319S, in the following exemplary reaction mix (20 µl): ssDNA/RNA Substrate 20 pmol (1 pmol/ul), 5′ App DNA Oligonucleotide 40 pmol (2 pmol/µl), 10X NEBuffer 1 (2 µl), 50 mM $MnCl_2$ (for ssDNA ligation only) (2 µl), Thermostable 5′ App DNA/RNA Ligase (2 µl (40 pmol)), and Nuclease-free Water (to 20 µl). The reaction can be incubated at 65°C for 1 hour. The reaction can be stopped by heating at 90°C for 3 minutes.

[00173] In some embodiments, the ligation reaction can be conducted using a T4 RNA ligase 2, *e.g.*, the T4 RNA ligase 2 from New England Biosciences, M0239S, in the following exemplary reaction mix (20 µl): T4 RNA ligase buffer (2 µl), enzyme (1 µl), PEG (10 µl), DNA (1 µl), Adapter (2 µl), and water (4 µl). The reaction can be incubated at 25°C for 16 hours. The reaction can be stopped by heating at 65°C for 20 minutes.

[00174] In some embodiments, the ligation reaction can be conducted using a T4 RNA ligase 2 Truncated, *e.g.*, the T4 RNA ligase 2 Truncated from New England Biosciences, M0242S, in the following exemplary reaction mix (20 µl): T4 RNA ligase buffer (2 µl), enzyme (1 µl), PEG (10 µl), DNA (1 µl), Adapter (2 µl), and water (4 µl). The reaction can be incubated at 25°C for 16 hours. The reaction can be stopped by heating at 65°C for 20 minutes.

[00175] In some embodiments, the ligation reaction can be conducted using a T4 RNA ligase 2 Truncated K227Q, *e.g.*, the T4 RNA ligase 2 Truncated K227Q from New England

Biosciences, M0351S, in the following exemplary reaction mix (20 µl): T4 RNA ligase buffer (2 µl), enzyme (1 µl), PEG (10 µl), DNA (1 µl), Adenylated Adapter (0.72 µl), and water (5.28 µl). The reaction can be incubated at 25°C for 16 hours. The reaction can be stopped by heating at 65°C for 20 minutes.

[00176] In some embodiments, the ligation reaction can be conducted using a T4 RNA ligase 2 Truncated KQ, *e.g.*, the T4 RNA ligase 2 Truncated KQ from New England Biosciences, M0373S, in the following exemplary reaction mix (20 µl): T4 RNA ligase buffer (2 µl), enzyme (1 µl), PEG (10 µl), DNA (1 µl), Adenylated Adapter (0.72 µl), and water (5.28 µl). The reaction can be incubated at 25°C for 16 hours. The reaction can be stopped by heating at 65°C for 20 minutes.

[00177] In some embodiments, the ligation reaction can be conducted using a T4 DNA ligase, *e.g.*, the T4 DNA ligase from New England Biosciences, M0202S, in the following exemplary reaction mix (20 µl): T4 RNA ligase buffer (2 µl), enzyme (1 µl), PEG (10 µl), DNA (1 µl), Adenylated Adapter (0.72 µl), and water (5.28 µl). The reaction can be incubated at 16°C for 16 hours. The reaction can be stopped by heating at 65°C for 10 minutes.

[00178] The second strand synthesis step can be conducted using any suitable enzyme. For example, the second strand synthesis step can be conducted using Bst polymerase, *e.g.*, New England Biosciences, M0275S or Klenow fragment (3'->5' exo-), *e.g.*, New England Biosciences, M0212S.

[00179] In some embodiments, the second strand synthesis step can be conducted using Bst polymerase, *e.g.*, New England Biosciences, M0275S, in the following exemplary reaction mix (10 µl): water (1.5 µl), primer (0.5 µl), dNTP (1 µl), ThermoPol Reaction buffer (5 µl), and Bst (2 µl). The reaction can be incubated at 62°C for 2 minutes and at 65°C for 30 minutes. After the reaction, the double stranded DNA molecules can be further purified.

[00180] In some embodiments, the second strand synthesis step can be conducted using Klenow fragment (3'->5' exo-), *e.g.*, New England Biosciences, M0212S, in the following exemplary reaction mix (10 µl): water (0.5 µl), primer (0.5 µl), dNTP (1 µl), NEB buffer (2 µl), and exo- (3 µl). The reaction can be incubated at 37°C for 5 minutes and at 75°C for 20 minutes. After the reaction, the double stranded DNA molecules can be further purified.

[00181] After the second strand synthesis, but before the first or semi-targeted PCR, the double stranded DNA can be purified. The double stranded DNA can be purified using any

suitable technique or procedure. For example, the double stranded DNA can be purified using any of the following kits: Zymo clean and concentrator, Zymo research, D4103; Qiaquick, Qiagen, 28104; Zymo ssDNA purification kit, Zymo Research, D7010; Zymo Oligo purification kit, Zymo Research, D4060; and AmpureXP beads, Beckman Coulter, A63882: 1.2x-4x bead ratio.

[00182] The first or semi-targeted PCR can be conducted using any suitable enzyme or reaction conditions. For example, the polynucleotides or DNA strands can be annealed at a temperature ranging from about 52°C to about 72°C, *e.g.*, at about 52°C, 53°C, 54°C, 55°C, 56°C, 57°C, 58°C, 59°C, 60°C, 61°C, 62°C, 63°C, 64°C, 65°C, 66°C, 67°C, 68°C, 69°C, 70°C, 71°C, or 72°C, or any subrange thereof. The first or semi-targeted PCR can be conducted for any suitable rounds of cycles. For example, the first or semi-targeted PCR can be conducted for 10-40 cycles, *e.g.*, for 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 cycles. The primer pool can be used at any suitable concentration. For example, the primer pool can be used at a concentration ranging from about 5 nM to about 200 nM, *e.g.*, at about 5 nM, 6 nM, 7 nM, 8 nM, 9 nM, 10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 60 nM, 70 nM, 80 nM, 90 nM, 100 nM, 110 nM, 120 nM, 130 nM, 140 nM, 150 nM, 160 nM, 170 nM, 180 nM, 190 nM, or 200 nM, or any subrange thereof.

[00183] The first or semi-targeted PCR can be conducted using any suitable temperature cycle conditions. For example, the first or semi-targeted PCR can be conducted using any of the following cycle conditions: 95°C 3 minutes, (95°C 15 seconds, 62°C 30 seconds, 72°C 90 seconds) x3 or x5, or (95°C 15 seconds, 72°C 90 seconds) x23 or x 21, 72C 1 minute, 4°C forever.

[00184] In some embodiments, the first or semi-targeted PCR can be conducted using KAPA SYBR FAST, *e.g.*, KAPA biosciences, KK4600, in the following exemplary reaction mix (50 µl): DNA (2 µl), KAPASYBR (25 µl), Primer Pool (26nM each) (10 µl), Aprimer (100uM) (.4 µl), and water (12.6 µl). The first or semi-targeted PCR can be conducted using any of the following cycle conditions: 95°C 30 seconds, (95°C 10 seconds, 50-56°C 45 seconds, 72°C 35 seconds) x40.

[00185] In some embodiments, the first or semi-targeted PCR can be conducted using KAPA HiFi, *e.g.*, KAPA Biosciences, KK2601, in the following exemplary reaction mix (50 µl): DNA (15 µl), KAPAHiFi (25 µl), Primer Pool (26nM each) (10 µl), and Aprimer (100uM) (0.4 µl).

The first or semi-targeted PCR can be conducted using any of the following cycle conditions: 95°C 3 minutes, (98°C 20 seconds, 53-54°C 15 seconds, 72°C 35 seconds) x15, 72°C 2 minutes, 4°C forever.

[00186] Bisulfite conversion can be conducted using any suitable techniques, procedures or reagents. In some embodiments, bisulfite conversion can be conducted using any of the following kits and procedures provided in the kit: EpiMark Bisulfite Conversion Kit, New England Biosciences, E3318S; EZ DNA Methylation Kit, Zymo Research, D5001; MethylCode Bisulfite Conversion Kit, Thermo Fisher Scientific, MECOV50; EZ DNA Methylation Gold Kit, Zymo Research, D5005; EZ DNA Methylation Direct Kit, Zymo Research, D5020; EZ DNA Methylation Lightning Kit, Zymo Research, D5030T; EpiJET Bisulfite Conversion Kit, Thermo Fisher Scientific, K1461; or EpiTect Bisulfite Kit, Qiagen, 59104.

[00187] In some embodiments, DNA molecules can be prepared using the procedures illustrated in Example 4, including the steps for constructing single-stranded polynucleotide, conversion of single-stranded polynucleotide library to double-stranded polynucleotide library, semi-targeted amplification of double-stranded polynucleotide library, and construction of sequence library. Such DNA molecules can further be analyzed for methylation status using any suitable methods or procedures.

I. Examples.

Example 1

[00188] In this example, the templates (*e.g.*, polynucleotides to be sequenced) are short DNA fragments less than about 200 bp long. These DNA fragments can include extracted DNA from plasma, enzyme-treated (*e.g.*, by a fragmentase) genomic DNA, or physically sheared DNA. The physically sheared DNA may be end repaired. In particular aspects, the template has a 3' hydroxyl group for ligation.

[00189] Typically, 10-30 ng of the properly prepared template DNA was dephosphorylated, for example, using 1 U FastAP Thermosensitive Alkaline Phosphatase (Thermo Scientific) in 100 mM MOPS (pH 7.5), 20 mM KCl, 10 mM $MgCl_2$, 2 mM DTT, and 5 mM $MnCl_2$ at 37°C for 10 minutes. The DNA was then denatured, for example, at 95°C for 2 minutes and put on ice for 1 minute.

[00190] A single-stranded adapter was synthesized from IDT with a 5' phosphate group and a 3' carbon spacer. The 5' end contains GA following by a 12-mer unique molecular identifier (UMI) sequence. A typical single-stranded adapter has the following sequence: /5Phos/GANNNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG/3SpC3/ ("5Phos" represents a 5' phosphate group, "NNNNNNNNNNNN" represents a 12-mer UMI sequence, and "3SpC3" represents a 3' carbon spacer.

[00191] A ligation reaction was then performed using the dephosphorylated, single-stranded DNA as template. The following final concentrations were used in the ligation reaction: 50 mM MOPS (pH 7.5), 10 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, and 2.5 mM $MnCl_2$, 50% PEG 4000, 0.5 μM adapter, 125 μM ATP, and 200 U Epicentre Circligase™. The reaction was incubated at 60°C for 2 hours, 80°C for 10 minutes, 85°C for 2 minutes, and held at 4°C.

[00192] The DNA was then double-stranded by adding the previous reaction volume to the following: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 1.25 U Taq DNA Polymerase (NEB), 1 μM reverse-complement primer (a primer that is reverse-complement to the adpator), and 200 μM dNTP mix. The reaction was incubated at 95°C for 30 seconds, 62°C for 2 minutes, 68°C for 10 minutes, and held at 4°C. A typical reverse-complement primer comprises the sequence set forth in SEQ ID NO: 3: CACTCTTTCCCTACACGACGC (5' to 3'). The following is an alignment between the adaptor and the reverse-complement primer:

```
Adaptor /5Phos/GANNNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG/3SpC3/
                                  :::::::::::::::::::::
Primer                            CGCAGCACATCCCTTTCTCAC
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[00193] The reaction was then purified using 1.6 (bead ratio) × AmPure® XP beads. The beads were added and incubated for 10 minutes. The mixtures were then transferred to a magnet for 5 minutes. The supernatant was then removed. The beads were washed 2 × with 150 μL 80% ethanol for 30 seconds each. All residual ethanol was then removed and the beads were dried for 3 minutes at room temperature off of the magnet. 15 μl of Low TE buffer (Thermo Fisher) was added to the beads and incubated for 2 minutes. The beads were then returned to the magnet for 1 minute. The supernatant was removed and stored for the next reaction. In one aspect, the bead ratio causes size selectivity in the purification process, and a bead ratio (such as 1.6 ×) can be selected that removes molecules shorter than about 100 bp.

[00194] A set of PCR primers were designed to minimize primer-primer interactions and off-target annealing. The primers were further optimized to land within close proximity to specific

variants. Once designed, the primers were synthesized by IDT. The primers were mixed in equal volume ratios into a primer pool. A semi-targeted PCR reaction was performed with the following reagents: all purified DNA from previous reaction, 1 × KAPA 2G multiplex master mix, 66 nM of each primer from pool, and 800 nM reverse-complement primer. The reaction underwent the following thermo-cycling program: 95°C 3 minutes, (95°C 15 seconds, 72°C 90 seconds) × 20, 72°C 1 minute, and held at 4°C.

[00195] The reaction was then purified using 1.6 (bead ratio) × AmPure® XP beads. The beads were added and incubated for 10 minutes. The mixtures were then transferred to a magnet for 5 minutes. The supernatant was then removed. The beads were washed 2 × with 150 µL 80% ethanol for 30 seconds each. All residual ethanol was then removed and the beads were dried for 3 minutes at room temperature off of the magnet. 20 µl of Low TE buffer (Thermo Fisher) was added to the beads and incubated for 2 minutes. The beads were then returned to the magnet for 1 minute. The supernatant is removed and stored for the next reaction. A bead ratio (such as 1.6 ×) can be selected that removes molecules shorter than about 100 bp, including free adaptor molecules, free primer molecules, and/or adaptor/primer dimers.

[00196] Another PCR reaction was then completed to add full length sequencing adapters and sample specific barcodes. The PCR reaction contained the following: 2 µL purified DNA from previous reaction, 1 × NEB ultra Q5 II master mix, 400 nM universal primer, and 400 nM barcode specific primer. The reaction underwent the following thermo-cycling program: 95°C 3 minutes, (98°C 10 seconds, 65°C 75 seconds) × 10, 65°C 2 minute, and held at 4°C.

[00197] The reaction was then purified using 0.8 (bead ratio) × AmPure® XP beads. The beads were added and incubated for 10 minutes. The mixtures were then transferred to a magnet for 5 minutes. The supernatant was then removed. The beads were washed 2 × with 150 µL 80% ethanol for 30 seconds each. All residual ethanol was then removed and the beads were dried for 3 minutes at room temperature off of the magnet. 25 µl of Low TE buffer (Thermo Fisher) is added to the beads and incubated for 2 minutes. The beads were then returned to the magnet for 1 minute. The supernatant is removed and is ready for sequencing. A bead ratio (such as 0.8 ×) can be selected that removes a majority of molecules shorter than about 200 bp.

Example 2

[00198] In this example, both genomic DNA (gDNA) samples with known variants and plasma samples are tested, using 10 ng and 20 ng inputs. The gDNA samples contained single nucleotide variations (SNVs, used interchangeably with "single nucleotide changes," SNCs), indels, CNVs, and fusions. Each variant was called at various allele fractions: 5%, 1%, 0.5%, and 0.1%. The sensitivity and specificity at each allele fraction for each mutation type were measured. The primer pool used here is shown in Table 1. Each target-specific primer can be used at the same volume ratio for the entire pool, or at a different volume ratio. For example, for a primer with volume ratio 2, that primer is added at 2 × volume of a primer with ratio 1.

[00199] Table 1:

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:4	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAACATCCCACGCCTAGTCCCTGG
SEQ ID NO:5	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACAGGTTTCCAGTGCCAGCT
SEQ ID NO:6	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACCCATAGAAGGGGTATTTGTTGGATTATTT
SEQ ID NO:7	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGCCACCTCCTTACTTTGCCTCCT
SEQ ID NO:8	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACAGATTGTGAACAGCCTTGGAAGCC
SEQ ID NO:9	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACATGCAGAAGTCCAGGCTGAAAAGG
SEQ ID NO:10	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACCATCATGATGTGTTACCCAGAATGTTTT
SEQ ID NO:11	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACCGTAGTTCACATGCACTCCTGT
SEQ ID NO:12	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTAACAGGTTAAGTGCTCCCAGGGG
SEQ ID NO:13	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTTTGTGTCGCTACCTCAGTTTGCC
SEQ ID NO:14	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGAGTAATTCACACAAGCTCACCTGA
SEQ ID NO:15	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCAGGATCTCAGGTCTCTCAAAGGG
SEQ ID NO:16	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGGCAAACACATCCACCCAAAGACTC
SEQ ID NO:17	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTGAATTGCAGTCCTTCCCCTCTG
SEQ ID NO:18	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATCTATTGTGGGCTCTGGGAATCCTG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:19	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATTCTTAAGTAATACTAACCTTGAACCGACTGGT
SEQ ID NO:20	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAAGAGCAGAAAGTCAGTCCCATGGA
SEQ ID NO:21	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAAGTTGGAAATTTCTGGGCCATGAA
SEQ ID NO:22	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACAGAAAGGGCCCAAATTCACCAAT
SEQ ID NO:23	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGAAGACCTCACATGCCACAAAGAA
SEQ ID NO:24	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATGTGGAGTGAACGTTGTTGGACTC
SEQ ID NO:25	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAACTCCATAAACTAAACAGAAAGCGGT
SEQ ID NO:26	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCGGGATTATGTCTCTTGTTTGGGGA
SEQ ID NO:27	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGGAATATAAGCTGGTGGTGGTGGG
SEQ ID NO:28	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGGGAGAAAATAGCACCTCACTTCCA
SEQ ID NO:29	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTAGGTCAGCTGAAGATCCTGTGAGC
SEQ ID NO:30	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCCACACGCAAATTTCCTTCCACTC
SEQ ID NO:31	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGCTGGCTGATCTATGTCCCTGAAG
SEQ ID NO:32	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGGTTTCCAACAGGTTCTTGCTGGTGT
SEQ ID NO:33	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTACTGCAGCTGTTTTCACCTCTGT
SEQ ID NO:34	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAAAAGTTTGCTGAGCTGGGTA
SEQ ID NO:35	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAGGTGTGTCTTTAATTGAAGCATGA
SEQ ID NO:36	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACAATAAAAGGCAGCTTGGACACGG
SEQ ID NO:37	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACCATAACCCACCACAGCTAGAACT
SEQ ID NO:38	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACCCAACACAACTTCCTTATGATCACAA
SEQ ID NO:39	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGTGCCAGCTGATGAAGACGGAG
SEQ ID NO:40	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATAATGACTCACCTGGGGCCACATT
SEQ ID NO:41	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATTGTCGTCGATTCTTGTGTGCTGT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:42	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAAGTTCTTCATCAGCTGTACTCCT
SEQ ID NO:43	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCACTTACCTGTGACTCCATAGAAAATCT
SEQ ID NO:44	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCACAAAACTTACAGATGCAGCAG
SEQ ID NO:45	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCAGCCCGAAGTCTGTAATTTTGAC
SEQ ID NO:46	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCTCATGTCTGAACTCAAAGTCCT
SEQ ID NO:47	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTGATTTGATGGAGTTGGACATGGC
SEQ ID NO:48	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAAATCAAAGAACCTGTGGCCAAAC
SEQ ID NO:49	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGATCTTTTCTTCACGGTTGCCTACT
SEQ ID NO:50	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGATGAACAGGAAGAAGCCCACCC
SEQ ID NO:51	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGGAGGTCAAATAAGCAGCAGGAGAA
SEQ ID NO:52	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTCACTGATGGAGGAGGTCTTGC
SEQ ID NO:53	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTACTTACCCACTGAAAAGCACTTCCTGA
SEQ ID NO:54	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCAAGGTTGCTGATTTTGGTCTTG
SEQ ID NO:55	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCCTGATAGTTGCTAAGAACCGGT
SEQ ID NO:56	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCTCATGTACTGGTCCCTCATTGC
SEQ ID NO:57	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCTCTCTGCCTCAATAAGCCAACCA
SEQ ID NO:58	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTGTCTCACTGCCTCATCTCTCACC
SEQ ID NO:59	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCAGTCTGTCCAGCACTTCCATTGGG
SEQ ID NO:60	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCCATGAACTCCACATTTGCCTTGGG
SEQ ID NO:61	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTCCTTGGTGACCGCTCTGCATCTA
SEQ ID NO:62	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTCCAGACGCATTTCCACAGCTACAC
SEQ ID NO:63	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAACAGCACAGTGAAAGCCAGCCAC
SEQ ID NO:64	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAAGCCTCCAGTCGCCTCAGTAAAG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:65	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAACTTTATAA GATCCTGGCTATCCTGTGGA
SEQ ID NO:66	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACAAGTGTTA GCTCCTATTATCCTGTCCCT
SEQ ID NO:67	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACGGATGGGA GATTGAAGATTTCTGTTG
SEQ ID NO:68	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACTTGGAGGC CTTGCAGAAGAAGCT
SEQ ID NO:69	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGGGGACTG TAGATGGGTGAAAAGAGCA
SEQ ID NO:70	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCACCAATC ATGATGCCGGAGAAA
SEQ ID NO:71	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAAGGTTGCA CTTGTCCACGCA
SEQ ID NO:72	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACAGCAGCA CCGAGACGATGAAG
SEQ ID NO:73	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACTCTCTCC TCCCCACTGCTG
SEQ ID NO:74	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACTCTGGCC TACGTGTTTGTTTCC
SEQ ID NO:75	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCGACATGTC TTTCCCCACAATCAT
SEQ ID NO:76	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCTTTGCACC TGTTTTGTTGTGTAC
SEQ ID NO:77	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCCCTGGTA GCTCATCATCTGG
SEQ ID NO:78	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTCTCCGTG GATGCCTTCAAGATC
SEQ ID NO:79	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTCTCTGGAT CCCACACCTTTACCA
SEQ ID NO:80	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTTCTTCTT CTCATCGCGGGCTTG
SEQ ID NO:81	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATACCCTCTCA GCGTACCCTTGTCC
SEQ ID NO:82	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATATAGTTATC ACCATAAAATTGTCATAGCTAGACATG
SEQ ID NO:83	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCGTGTACTT CCGGATCTTCTGCTG
SEQ ID NO:84	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGCCTTTCAC GTTCCTTTCCCCAAA
SEQ ID NO:85	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTCTGGGAGC TTCATCTGGACCTGG
SEQ ID NO:86	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAATTGGCATG CTCTTCAATCACTGA
SEQ ID NO:87	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACACCTGTCA TGTAGCAGCTTTCAG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:88	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACACGACGGGAAGACAAGTTCATG
SEQ ID NO:89	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACACTTCTCCATTCTTCACAAGGGT
SEQ ID NO:90	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACATGCTCCCAGGCTGTTTATTTGA
SEQ ID NO:91	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTTCTCCAGGACCACGGACTG
SEQ ID NO:92	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGAGAGTTGCGGGGATTGAC
SEQ ID NO:93	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGGAATGTGGGGCCAGAC
SEQ ID NO:94	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGGTTACATACTTGGACTTGGT
SEQ ID NO:95	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATAGCTGACACCACGATACTTGACA
SEQ ID NO:96	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGTAACAAACCTTCACGTCCTGCA
SEQ ID NO:97	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTGGCATGGGGAAATATAAACTTGTTTGA
SEQ ID NO:98	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGTTTAAGATTTGCCCAGACTCAGC
SEQ ID NO:99	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATCCTGCCAAAGTTTGTGATTCCA
SEQ ID NO:100	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATGTCTTTGCAGCCGAGGAG
SEQ ID NO:101	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCCTCCTTCTGGCCACCATGCG
SEQ ID NO:102	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGTAAATTTCTCATGGGCAGCTCC
SEQ ID NO:103	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTTGAAGCACTACACAGGCCACTT
SEQ ID NO:104	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGAAGATGATGTTCTCCAGGTCGAA
SEQ ID NO:105	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGTAGTAGGGGAAGATCATCTGCTGG
SEQ ID NO:106	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCAAAAGACTTGGTGTTGTTGATGGC
SEQ ID NO:107	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCATGGCAGGGCTCTAGGATGA
SEQ ID NO:108	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTGGTCAAGGTCACATTCTTCCA
SEQ ID NO:109	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGCGTCATCATCTTTGTCATCGTGT
SEQ ID NO:110	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTGCCCCTTAGCTGTGATTTCCTA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:111	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTGCTTCAACTAAATTTAACTGTCAGCA
SEQ ID NO:112	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCGTCCTCCTTCCTCACTCTGC
SEQ ID NO:113	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGATGTAGCTGTGCATGTCCTGGTG
SEQ ID NO:114	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCAGTGCTAACCAAGTTCTTTCTTTTGC
SEQ ID NO:115	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCCAGTCTGTATCACATCCACCTCAT
SEQ ID NO:116	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGACGCAACAGAGAAAGACTTGTCAG
SEQ ID NO:117	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGAGCAAACCCCTATGTCCACAAG
SEQ ID NO:118	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCACTCTGGATTGTGTACACTCTGTCAA
SEQ ID NO:119	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTGAGCGCTTCGAGATGTTCCGA
SEQ ID NO:120	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAAAGGAAATCACGCTGTCCCCTGTG
SEQ ID NO:121	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAACCCCAGTCAGCTCCAGAGTCAC
SEQ ID NO:122	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAACTCTCTTTGACTGCAGAATCCAACTGTAA
SEQ ID NO:123	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAATCACCACCCCACCCAATTCCAGG
SEQ ID NO:124	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTACGACAAGTGGGAGATGGAACGCA
SEQ ID NO:125	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAGGTGAGAGGCAGTGGTCAGGGTC
SEQ ID NO:126	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAACGAGTGCTTCATCAAGGTGCC
SEQ ID NO:127	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCACATTTCAGCAACAGCAGCATCT
SEQ ID NO:128	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGATCTTGTAGGGGATGTTGAGGCT
SEQ ID NO:129	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTACAACCCCACCACGTACCAGATG
SEQ ID NO:130	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTCCTTTTCCTCCTCTTCTCCTGGC
SEQ ID NO:131	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTCTCAATGGCTTCTGTCCTGTGGA
SEQ ID NO:132	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGAATCCAGTGTTTCTTTTAAATACCTGTTAAG
SEQ ID NO:133	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGCATTCTGGGAGCTTCATCTGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:134	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGCCTGTTCTTTCCAAGGGTGC
SEQ ID NO:135	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGGGAATGAAAGTGGGATCAGGGA
SEQ ID NO:136	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGATAAGGTTAAGGGCCCCAACGGTA
SEQ ID NO:137	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGAAGTCGATCACCTGCCTCACTAT
SEQ ID NO:138	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGATGCTGCACAGGTGTACAATCC
SEQ ID NO:139	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGTGCTCATTTAGTCCTGGGGCAG
SEQ ID NO:140	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGTTTGGGGAAGAGTGGGCTAGTG
SEQ ID NO:141	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTAGACTACCGAGCTACTTTTCCAGAAGGTA
SEQ ID NO:142	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGCCGAGTATCCTGGAGCCTC
SEQ ID NO:143	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGTGACTATCTCCCTGGGTGTAGC
SEQ ID NO:144	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCACACTCTGAGGCGGAACATG
SEQ ID NO:145	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCCAGAGACATTGCTGCCAGAAAC
SEQ ID NO:146	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTTCACGCTCCTTCCCTATCCCTT
SEQ ID NO:147	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGTCCTGCTTGCTTACCTCGCTTAG
SEQ ID NO:148	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTATCTGTATCAAAGAATGGTCCTGCACC
SEQ ID NO:149	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTCTCGGTTCTCTGATTCCTGGCAG
SEQ ID NO:150	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAATGTGAGCCCTTGAGATCTGCGG
SEQ ID NO:151	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAACAATGCCTCCACGACCATCATCAG
SEQ ID NO:152	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGACCCAAGCTGCCTGACCC
SEQ ID NO:153	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGCAACCCACAGATGTTCCCGG
SEQ ID NO:154	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGCCCCTTTCTTTGTTCAGCCCC
SEQ ID NO:155	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGTGCCCTTGGTTCGGACAGACAAC
SEQ ID NO:156	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAATCTCCTCCCAACTCAACTTCCCAG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:157	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACACCTAGCTG TGATCCTGAAACTGAATTT
SEQ ID NO:158	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACCCAAACAAA AGCGATCTCCTCCAG
SEQ ID NO:159	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTCACCGGTG GATGAAGTGGTTTTC
SEQ ID NO:160	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTTCTCCTCC ACAAATCCAGAGCTG
SEQ ID NO:161	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCCTTTCCCT CTGCCCTTTTCAAG
SEQ ID NO:162	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTCCCCACC CCCTGATCAG
SEQ ID NO:163	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGAGGCCATC TTCCATCTTCTCACA
SEQ ID NO:164	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTCTTCCCAC AAGTTCGCTCTTTGG
SEQ ID NO:165	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTGGGCATTG TATGGAAACTGAGGC
SEQ ID NO:166	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATACGGCCAGG CATTGAAGTCTCATG
SEQ ID NO:167	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATAGTTTCTGA AGGAATGCTATGGTATGAAACA
SEQ ID NO:168	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCCAGCCAGA CCCAGCCAGTATTAT
SEQ ID NO:169	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCTTGAAGGC ATCCACGGAGACC
SEQ ID NO:170	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAGGAGAAG ACAAGAGGAGACAGAGTC
SEQ ID NO:171	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAACACCCAGC CCTCGGTAAG
SEQ ID NO:172	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCAACGGAC ATGAGTTTGTTTTCC
SEQ ID NO:173	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCACCGAGA CGATGAAGGAGAAG
SEQ ID NO:174	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGGACCCGAC AAAACCTAAAGATGG
SEQ ID NO:175	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCATCCCTGA CTGTGAGATCAAGAA
SEQ ID NO:176	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCCAATTGCA GGTAAAACAGTCAAG
SEQ ID NO:177	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCGTATTTAC TGCCGTTCTTTTCCA
SEQ ID NO:178	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCCCAGCAA TTTCCTCCCTTGTT
SEQ ID NO:179	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTGTGTTCTG CCCCCATTTC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:180	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGAAGAATGTGTCAGCCTCAAAGAA
SEQ ID NO:181	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTGCCTGCCAATGGTGATG
SEQ ID NO:182	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTAAGGGGCACAGGGTAGGTAGT
SEQ ID NO:183	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTAATGTTAAGAATGTACTGATATTTATTACTGAACCTTTAGGT
SEQ ID NO:184	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTATTTAAGATTACGAAGGTATTGGTTTAGACAGAAAT
SEQ ID NO:185	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCGGGTTGGCTCTAAAGTAGTCCT
SEQ ID NO:186	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCCACTTCTACGACTTCTTCAACC
SEQ ID NO:187	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGGAAAGGGACGAACTGGTGTAATG
SEQ ID NO:188	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGCAAAAATCCAGTAGTAGCTAGCTCTGC
SEQ ID NO:189	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGGATCATATTGGCCTGTCTGCTC
SEQ ID NO:190	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAATAGGTTTCATGGACTCAGTTACTACCTG
SEQ ID NO:191	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGCCAAGCCCAGTTCTGGAAG
SEQ ID NO:192	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACAGTTGATACAAAACAAGCCCACG
SEQ ID NO:193	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGCAGATCAAACGGGTGAAGGACTC
SEQ ID NO:194	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGTCCAGGAAATGATATCACATAAGT
SEQ ID NO:195	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATACTTACGCGCCACAGAGAAGTTG
SEQ ID NO:196	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATATATTCCAGTGGTTTGTTGCTCTCTG
SEQ ID NO:197	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATGCCTTATTGCGACAGATCCGGA
SEQ ID NO:198	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATGTTCTGGAAGGCAAACTCCATGG
SEQ ID NO:199	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTCTGATTCCTCACTGATTGCTCT
SEQ ID NO:200	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTGCTGAAAATGACTGAATATAAACTTGTGG
SEQ ID NO:201	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTGGATCCTGAACTGGGCAAAATTA
SEQ ID NO:202	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTTCACAGACATCCTTGCACATCTC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:203	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTGAACATACTAAATGCTCCAGT
SEQ ID NO:204	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGTTCAGCAAATCTTCTAATCCATGAGG
SEQ ID NO:205	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTACTCTGTCTCGTCAATGTCCAGCA
SEQ ID NO:206	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAGATTCCAGTTCTTGTGTGCGTGC
SEQ ID NO:207	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCCCTCCCACAGTTGCTTCAAGT
SEQ ID NO:208	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGGGAAGAACAGCCTAGACTTGGG
SEQ ID NO:209	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCCAGGCTTGCTGTAATTACCCAG
SEQ ID NO:210	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAATCCCCAACCCAATAGACCCACCC
SEQ ID NO:211	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGAGTTCAAGTACTGGGGGCCA
SEQ ID NO:212	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGCAGAGAACCAAGCCCTCCTAAG
SEQ ID NO:213	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTGAGCCTGTTTTGTGTCTACTGTTTC
SEQ ID NO:214	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTGGGATTGCAGATTGGGCCTTG
SEQ ID NO:215	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCCATACCCTCTCAGCGTACC
SEQ ID NO:216	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAAGGACCAAGGAGCAGAGGAGG
SEQ ID NO:217	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGACCGAGGACAACGTGATGAAGATC
SEQ ID NO:218	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGCAGGTGGAAGTAGGAGGTCTTG
SEQ ID NO:219	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGATAGGAAGCTGTGGAGTGATGAGC
SEQ ID NO:220	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCAATTCAGACCCCAACAGTACGAA
SEQ ID NO:221	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCAGAGTAGTAGCTGCAAATAATCTAGGGTTTGG
SEQ ID NO:222	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCATCGGGACTTGGCAGCCA
SEQ ID NO:223	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGGGACCACACTGAGTTCTCTGT
SEQ ID NO:224	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGGTGCGGGAGTGAATAGGCC
SEQ ID NO:225	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTACTTGAAGGCCTCCGGAATGCG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:226	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCTTTCCTCTGTGTTGGCGGATACC
SEQ ID NO:227	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGGCCTTGGTGTGCATTCTTCTCTCT
SEQ ID NO:228	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTCGGTCATGATGGTCGAGGTGC
SEQ ID NO:229	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAACTGCAGAGTATTTGGGCGAATG
SEQ ID NO:230	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAGGTTGGAATGAGCTGGATAAGGC
SEQ ID NO:231	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACATTTCTTTGACCATTTGTTTTGCTGT
SEQ ID NO:232	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCAAAGCAGAAGTAAAACCAGATGC
SEQ ID NO:233	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCTTACACACACGCAAAATACTCCT
SEQ ID NO:234	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTCCCCTGTCATCCTCACACTTTTC
SEQ ID NO:235	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGTCATAGTTGTTGCAAGCCGAAGAG
SEQ ID NO:236	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGTGGAGAACAAGTTTGGCAGCATC
SEQ ID NO:237	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACCTTGTAGCCTCCAATGCGATGC
SEQ ID NO:238	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCAACTAAAAGCTTCTGTCTGCAAG
SEQ ID NO:239	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCCCCAATAATAATCAGCCACCCCC
SEQ ID NO:240	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTGGATGGAACTGATGTCTGGACG
SEQ ID NO:241	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGCAGCAAATTCAACCACCAGAACA
SEQ ID NO:242	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGCATTTGAAGTTTTTATTAGTGATGGATTTG
SEQ ID NO:243	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGTCTGAAGTGAGAACTCCGTGTGG
SEQ ID NO:244	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATAACGACACAACACAAAATAGCCGT
SEQ ID NO:245	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCAAGAACCCAGACCTCGAGTTTG
SEQ ID NO:246	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCATACATTCGAAAGACCCTAGCCT
SEQ ID NO:247	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCCGTGATGTTCCATGTAATACTGG
SEQ ID NO:248	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTTCTAGAACAGTAGACACAAAACA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:249	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTAGGAAAGAGGCAAGGAAAGGTGA
SEQ ID NO:250	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTCTTTTCACCCATCTACAGTCCCC
SEQ ID NO:251	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGAGGAAGTGGATTTTGCAGGTTG
SEQ ID NO:252	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAATATATCCACTCAATCTTCTACTTTAAAATGACTTAGG
SEQ ID NO:253	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGACATGAAGCAGGCTGATACTACACA
SEQ ID NO:254	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCACATTCCATTCTTACCAAACTCT
SEQ ID NO:255	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAGTTCCCATCTCAGGCTGG
SEQ ID NO:256	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCCCCGCCTCTGAATATTTCTTTAA
SEQ ID NO:257	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGCTACAAGAACTACCGATACCGTG
SEQ ID NO:258	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTACATGGCCACTCAGATCTCGTCAG
SEQ ID NO:259	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCACCACATTACATACTTACCATGCC
SEQ ID NO:260	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCAGTGGGGAACAAGAAGTGGAGAA
SEQ ID NO:261	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCCATCAGCCTCCAGTTCAGCAAG
SEQ ID NO:262	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCTGAACACTTCTTCCAGGTCCAAG
SEQ ID NO:263	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGATTTATTCTTTCAACAGCCACGG
SEQ ID NO:264	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCTCAGTTCCCTCCTCTATGCAAT
SEQ ID NO:265	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGGTTGGTCAGAAAGATAAGCCAGT
SEQ ID NO:266	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGTCACATTATAAAGATTCAGGCAAT
SEQ ID NO:267	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTATCACTGTCTGTCTCTCCTGCAGCC
SEQ ID NO:268	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTATTTGAGCTAGAACCAGTGCCAGGC
SEQ ID NO:269	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCCCCAATCTACCTGTGTCAGTTCCC
SEQ ID NO:270	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTACCTGCAGTAAAACGATGTTTGC
SEQ ID NO:271	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTCCAGGCGTTTCTTCCATCCTTCC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:272	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTCTCTGGCAGGTCATGATGATGGG
SEQ ID NO:273	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTTCATACCAGGACCAGAGGAAACC
SEQ ID NO:274	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTCTTCCTTTTCCATGCAGTGTGTCCA
SEQ ID NO:275	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTAAACATGCATTGATAACACCCCTACTGCT
SEQ ID NO:276	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTCTAATCACATAGGCAGGAAATCTCAGTG
SEQ ID NO:277	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTCTTCCCTCCCCTCGAAATGAAGCT
SEQ ID NO:278	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTGTTTCTCCCACACAGACACTATTGTG
SEQ ID NO:279	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTTGTGTGAGTGAATGTGTGCCAGGG
SEQ ID NO:280	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAGCTCTTCCTGTTTCAGTCCCCATT
SEQ ID NO:281	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTTTTCTTATGTGCTTTTAGGGCCCA
SEQ ID NO:282	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTTCTTCCCATGATGATCTGTCCCT
SEQ ID NO:283	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGCTGTGAAGGTAAGAAGTGGCTC
SEQ ID NO:284	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCGCTGTAGAACGCACCATAGAAGC
SEQ ID NO:285	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTCTGCTGGTCGTGGTCTTGGG
SEQ ID NO:286	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAAGAGAGCAACACCCACACTTACAC
SEQ ID NO:287	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGTATGGTCATGGAAGGGGCTT
SEQ ID NO:288	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGGAAGGTGAAGGTGCTTGGATCT
SEQ ID NO:289	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGATGTGATGAGAGGTGGATGGGTAG
SEQ ID NO:290	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCAAGAGTCCCAACCATGTCAAAAT
SEQ ID NO:291	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCTTCTGATACGATCTGTGACCTGT
SEQ ID NO:292	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTATGCGACACTTACAGCTGCCCAG
SEQ ID NO:293	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTCTGATGTACCAACCTCACCAACA
SEQ ID NO:294	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAGTGCCCGAAGTGTAAGCCCAACTA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:295	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTCCTCTTCCTACAGTACTCCCCT
SEQ ID NO:296	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGCCTGTCCTCATGTATTGGTCTCTC
SEQ ID NO:297	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAGTAACTCAGCAGCATCTCAGGGC
SEQ ID NO:298	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAGCAATTTAGGTATGAAAGCCAGC
SEQ ID NO:299	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAGCCAGAAATATCCTCCTTACTCA
SEQ ID NO:300	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTTCCATCCTCTGCTGTCACCTCTTG
SEQ ID NO:301	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTTTTCTTCCCTTTAGATGCTCTGCT
SEQ ID NO:302	2	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTTTAACCATGCAGATCCTCAGTTTGTG
SEQ ID NO:303	3	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCCTTGTCTCTGTGTTCTTGTCCCC
SEQ ID NO:304	3	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCAATATTGTCTTTGTGTTCCCGGA
SEQ ID NO:305	3	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCATATCCTCCTCTTTCTGCCCAGGG
SEQ ID NO:306	3	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGAAGTCCTCGTTGTCTTGTTGGCA
SEQ ID NO:307	4	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACCATCTCACAATTGCCAGTTAACG

[00200] In one aspect, the present method can achieve increased sensitivity at certain loci. In Table 2 below, the present method is compared to the conventional hybrid capture method. The present method calls several loci missed by hybrid capture, which is directly related to the increased conversion rate of the present method.

[00201] Table 2:

Sample	Gene	Indel	Hybrid Capture Call	Call of the Present Method
Plasma 1	EGFR	p.E746_A750delELREA	NOT CALLED	0.235%
Plasma 2	EGFR	p.E746_A750delELREA	NOT CALLED	0.274%
Plasma 3	EGFR	p.E746_A750delELREA	NOT CALLED	0.324%
Plasma 4	EGFR	p.E746_A750delELREA	NOT CALLED	13.043%
Plasma 5	EGFR	p.E746_A750delELREA	NOT CALLED	16.195%
Plasma 6	EGFR	p.E746_A750delELREA	NOT CALLED	17.719%

Sample	Gene	Indel	Hybrid Capture Call	Call of the Present Method
Plasma 7	EGFR	p.E746_A750delELREA	0.44%;	1.468%

[00202] Extremely high ligation efficiencies of ~80% were achieved, resulting in conversion rates of ~60%, compared to ~25% and 10% in standard libraries, respectively. Exemplary conversion rates of the present methods are shown in Table 3.

[00203] Table 3:

Chromosome	Target Position	UMI Depth (out of 6666 input molecules) of the present example	Conversion Rate of the present example	Hybridization Capture UMI Depth (out of 20000 input molecules)	Hybridization Capture Conversion Rate
chr10	43609975	5322	0.79837984	433	0.02165
chr10	43609981	3184	0.47764777	435	0.02175
chr6	117647309	307	0.04605461	521	0.02605
chr6	117647319	400	0.060006	610	0.0305
chr1	115258623	165	0.02475248	684	0.0342
chr6	117657204	106	0.01590159	699	0.03495
chr3	178917032	358	0.05370537	952	0.0476
chr6	117658352	2071	0.31068107	1326	0.0663
chr6	117647426	2820	0.4230423	1432	0.0716
chr6	117657358	2353	0.3529853	1667	0.08335
chr3	178936004	446	0.06690669	1895	0.09475
chr6	117658053	1449	0.21737174	1921	0.09605
chr12	25398153	105	0.01575158	1929	0.09645
chr6	117658058	1584	0.23762376	1939	0.09695
chr6	117645959	1603	0.24047405	2128	0.1064
chr6	117658257	833	0.1249625	2204	0.1102
chr7	55221932	4199	0.62991299	2260	0.113
chr6	117647205	867	0.13006301	2271	0.11355
chr3	178927985	849	0.12736274	2363	0.11815
chr3	178927989	928	0.13921392	2377	0.11885
chr7	55228037	2474	0.37113711	2573	0.12865
chr12	25398295	2385	0.35778578	2575	0.12875
chr12	25398280	2391	0.35868587	2804	0.1402
chr6	117658112	3140	0.47104711	2815	0.14075
chr6	117645611	1712	0.25682568	2852	0.1426
chr6	117646015	2735	0.41029103	2912	0.1456
chr12	25378604	2168	0.32523252	2962	0.1481
chr1	115258783	3346	0.5019502	3025	0.15125
chr6	117646362	1105	0.16576658	3090	0.1545
chr6	117658134	379	0.05685569	3140	0.157
chr12	25378610	2300	0.3450345	3178	0.1589

Chromosome	Target Position	UMI Depth (out of 6666 input molecules) of the present example	Conversion Rate of the present example	Hybridization Capture UMI Depth (out of 20000 input molecules)	Hybridization Capture Conversion Rate
chr2	29436845	3089	0.46339634	3200	0.16
chr3	178928065	3037	0.45559556	3244	0.1622
chr3	178936100	1469	0.22037204	3284	0.1642
chr7	55228009	4204	0.63066307	3776	0.1888
chr17	7573999	3228	0.48424843	3789	0.18945
chr17	7579588	2047	0.30708071	3804	0.1902
chr17	7579584	4174	0.62616262	3969	0.19845
chr1	115252244	3395	0.50930093	4080	0.204
chr2	29443737	3636	0.54545455	4087	0.20435
chr7	116412017	5521	0.82823282	4214	0.2107
chr7	116423378	1939	0.29087909	4267	0.21335
chr17	7578291	4943	0.74152415	4302	0.2151
chr7	116412016	5560	0.83408341	4321	0.21605
chr3	178928280	2069	0.31038104	4411	0.22055
chr7	116423432	5859	0.87893789	4483	0.22415
chr3	178916923	2200	0.330033	4634	0.2317
chr17	7578238	1925	0.28877888	4636	0.2318
chr2	29445367	4446	0.6669667	4650	0.2325
chr6	117650705	2202	0.33033303	4735	0.23675
chr6	117650651	1396	0.20942094	4907	0.24535
chr6	117645786	2029	0.30438044	4965	0.24825
chr7	55211154	4479	0.67191719	4972	0.2486
chr7	116411968	148	0.02220222	5020	0.251
chr3	178916829	710	0.10651065	5091	0.25455
chr7	55229294	4929	0.73942394	5111	0.25555
chr7	55259556	4813	0.7220222	5131	0.25655
chr2	29432724	3421	0.51320132	5136	0.2568
chr8	38274906	3087	0.46309631	5259	0.26295
chr6	117647147	4329	0.64941494	5338	0.2669
chr6	117645662	3116	0.46744675	5546	0.2773
chr17	37881465	1101	0.16516652	5581	0.27905
chr17	37881366	4657	0.69861986	5595	0.27975
chr17	37879658	5429	0.81443144	5619	0.28095
chr17	7576927	167	0.02505251	5665	0.28325
chr7	55214422	5095	0.76432643	5682	0.2841
chr6	117646459	1940	0.2910291	5693	0.28465
chr17	37881381	4992	0.74887489	5717	0.28585
chr17	37868235	3763	0.56450645	5729	0.28645
chr6	117645719	879	0.13186319	5765	0.28825
chr2	29443680	4606	0.6909691	5840	0.292
chr7	55259490	3837	0.57560756	5920	0.296
chr17	7578544	2217	0.33258326	5960	0.298
chr7	55249105	4317	0.64761476	6011	0.30055

Chromosome	Target Position	UMI Depth (out of 6666 input molecules) of the present example	Conversion Rate of the present example	Hybridization Capture UMI Depth (out of 20000 input molecules)	Hybridization Capture Conversion Rate
chr2	29446552	1309	0.19636964	6025	0.30125
chr6	117646632	408	0.06120612	6265	0.31325
chr17	37879607	1530	0.22952295	6307	0.31535
chr6	117647088	1238	0.18571857	6396	0.3198
chr17	7577003	1285	0.19276928	6471	0.32355
chr17	37881412	5115	0.76732673	6550	0.3275
chr2	29447600	1022	0.15331533	6599	0.32995
chr2	29447605	946	0.14191419	6645	0.33225
chr6	117646875	1612	0.24182418	6684	0.3342
chr6	117646910	1777	0.26657666	6985	0.34925
chr2	29446439	898	0.13471347	7198	0.3599
chr6	117646732	1343	0.20147015	7210	0.3605
chr10	43611123	1142	0.17131713	7473	0.37365
chr10	43611821	2212	0.33183318	7600	0.38
chr10	43611334	1175	0.17626763	7786	0.3893
chr10	43611728	978	0.14671467	7794	0.3897
chr10	43610389	1202	0.18031803	7794	0.3897
chr10	43611251	1313	0.1969697	7918	0.3959
chr10	43611621	246	0.03690369	8053	0.40265
chr2	29447805	2223	0.33348335	8183	0.40915
chr2	29447732	728	0.10921092	8227	0.41135
chr10	43611971	535	0.08025803	8235	0.41175
chr10	43611006	1539	0.23087309	8241	0.41205
chr10	43611452	1239	0.18586859	8446	0.4223
chr10	43610636	1921	0.28817882	8476	0.4238
chr2	29447492	977	0.14656466	8498	0.4249
chr10	43610782	733	0.109961	8502	0.4251
chr2	29448196	589	0.08835884	8570	0.4285
chr2	29446294	2579	0.38688869	8956	0.4478
chr2	29447924	618	0.09270927	8977	0.44885
chr10	43610887	1922	0.28832883	9029	0.45145
chr2	29448061	591	0.08865887	9053	0.45265
chr10	43610529	777	0.11656166	9120	0.456
chr2	29448322	918	0.13771377	9435	0.47175

[00204] As shown in **FIG. 6**, the distribution displays that the conversion rate goes up significantly higher for the present method than for hybrid capture. While in this example, the conventional hybridization capture method may achieve a conversion rate of about 47%, the present method achieves a much higher conversion rate of about 88%. This significantly higher conversion rate, together with the ability to multiplex across hundreds of loci in a single

reaction, makes the present method ideal for high throughput and high accuracy sequencing and analysis of polynucleotides, especially for samples with very low allele fractions, such as ctDNA carrying cancer-associated SNPs and/or mutations.

[00205] Additionally, the on-target rate is up to 70% for very small target regions (~30,000 bases) resulting in enrichment factors of >40,000 ×, compared to an enrichment factor of ~2000 × for standard libraries. The improved efficiencies have resulted in greater sensitivities, allowing accurate calls down to 0.1% at many variants. SNVs, indels, CNVs, and fusions were accurately called. Furthermore, the procedure is very robust, with a failure rate of 0%.

Example 3

[00206] In this example, a method for constructing a library from extracted plasma DNA is described, for example, to interrogate single nucleotide changes (SNCs), indels, copy number variations (CNVs), and fusions, from circulating tumor DNA. As a principle in this example, extracted plasma DNA (*e.g.*, from human) is dephosphorylated and denatured. A single stranded DNA ligation adds a universal adapter to the 3' end of each molecule. The DNA then undergoes semi-targeted PCR using a site-specific primer and a reverse-complement primer to the adapter. Libraries are made with a secondary PCR to add full length adapters and barcodes to each molecule.

[00207] Equipment, materials, and supplies used in this example include: Veriti Thermocycler, 96 well magnet, 96 well ice block, Vortexer, Plate mini centrifuge, Semi-skirted 96 well PCR plate, Plate seals, Pipettes, and Pipette tips.

[00208] Reagents and media used in this example include: Nuclease free water (Ambion/Thermo: AM9939), Low TE buffer (Thermo fisher: 12090015), Circligase Kit (Epicenter: CL4115K), FastAP (Thermo Fisher: EF0651), 50% PEG 4000 (Sigma: 95904-250g-F. Dilute 5g in 10 mL in Nuclease free water Ambion/Thermo: AM9939), 10 μM N12 Adapter (IDT), Taq polymerasae (NEB: M0273S), dNTP mix (NEB: N0447L), Standard Taq buffer (NEB: M0273S), Ampure XP beads (Agincourt/Beckman Coulter: A63881), 100 uM Reverse complement primer (IDT), Primer mix (IDT), KAPA 2G multiplex (KAPA: KK5802), NEBNext Ultra Q5 II (NEB: M0544L), and 10 μM NEBNext Multiplex Oligos (IDT).

PROCEDURE

Dephosphorylation:

[00209] 1. Creating the master mix below:

Reagent	Volume (µL)
Water	To 22 µL
Circligase™ buffer	4
$MnCl_2$	2
FastAP	1
DNA	20 ng

2. Adding master mix and DNA to a 96 well plate.
3. Sealing the plate to briefly vortex it and spin down.
4. Running the following program: 37°C 10 min, 95°C 2 min.
5. Immediately after, placing the plate on a 96 well ice block for 1 min, then removing the plate to continue immediately with ligation below.

Ligation:

[00210] 1. Creating the master mix below:

Reagent	Volume (µL)
50% PEG 4000	8
Adapter	2
ATP	2
Water	4
Circligase™	2

2. Adding 18 µl of the master mix directly to the products from dephosphorylation.
3. Sealing the plate to briefly vortex it and spin down.
4. Running the following program: 60°C 2 hr, 80°C 10 min, 85°C 2min, 4°C on hold.
5. Proceeding immediately to second strand synthesis below.

Second Strand Synthesis:

[00211] 1. Creating the master mix below:

Reagent	Volume (µL)
Water	3.25
Primer	0.5
dNTP	1
Standard Taq buffer	5
Taq polymerase	0.25

2. Adding 10 µl of master mix directly to the ligation products.
3. Sealing the plate to briefly vortex and spin down.
4. Running the following program: 95°C 30s, 62°C 2 min, 68°C 10 min, 4°C on hold.
5. Proceeding immediately to AmPure® XP bead cleanup below.

AmPure® XP Bead Cleanup:

[00212] 1. Vortexing the AmPure® beads until the solution is homogeneous.
2. Adding 80 µl of beads to the products from second strand synthesis, and pipetting up and down to homogenize beads.
3. Incubating at room temp for 10 min.
4. Transferring plate to a magnet and incubating on the magnet for 5 min or until all of the beads have moved towards the magnet.
5. Removing all supernatant.
6. Adding 150 µL of 80% EtOH and incubating for 30 s.
7. Removing the supernatant.
8. Repeating steps 6-7.
9. Ensuring that all residual ethanol is removed and removing plate from the magnet and incubating at room temperature for 3 min.
10. Adding 16 µl of Low TE buffer and pipetting up and down to homogenize beads.
11. Incubating at room temperature for 2 min.
12. Transferring plate to a magnet and incubating on magnet for 1 min or until all of the beads have move to the magnet.
13. Removing 15 µl of the supernatant and placing it in a clean plate.
14. Proceeding with the 1st PCR below or store at -20°C.

1st PCR:

[00213] 1. Creating the master mix below:

Reagent	Volume (µL)
KAPA 2G multiplex master mix	25
Primer pool	10
Reverse primer	0.4
DNA	15

2. Adding 35 µl of master mix to 15 µl of purified DNA.

3. Sealing the plate to briefly vortex and spin it down.

4. Running the following program: 95°C 3min, (95°C 15s, 72°C 90s) × 20, 72°C 1min, 4°C on hold.

5. Proceeding immediately to AmPure® XP bead cleanup below.

AmPure® XP Bead Cleanup

[00214] 1. Vortexing the AmPure® XP beads until the solution is homogeneous.

2. Adding 80 µl of beads to the products from second strand synthesis and pipetting up and down to homogenize beads.

3. Incubating at room temperature for 10 min.

4. Transferring plate to a magnet and incubating on the magnet for 5 min or until all of the beads have moved towards the magnet.

5. Removing all supernatant.

6. Adding 150 µL of 80% EtOH and incubating for 30 s.

7. Removing supernatant.

8. Repeating steps 6-7.

9. Ensuring that all residual EtOH is removed and removing plate from the magnet and incubating at room temperature for 3 min.

10. Adding 20 µl of low TE buffer and pipetting up and down to homogenize beads.

11. Incubating at room temperature for 2 min.

12. Transferring plate to a magnet and incubating on magnet for 1 min or until all of the beads have move to the magnet.

13. Removing 19 µl of the supernatant and place it in a clean plate.

14. Proceeding with the 2nd PCR below OR store at -20°C.

2nd PCR

[00215] 1. Creating the master mix below, and note that the index primer and DNA are added separately, and the remaining DNA is stored at -20°C:

Reagent	Volume (µL)
NEBNext Q5 Ultra II master mix	25
Index primer	2
Universal primer	2
Water	19
DNA	2

2. In a new plate, adding 46 µL master mix, 2 µL index primer, and 2 µL DNA.

3. Sealing the plate to briefly vortex and spin it down.

4. Running the following program: 95°C 3 min, (98°C 10s, 65°C 75s) × 10, 65°C 2 min, 4°C on hold.

5. Proceeding immediately to bead cleanup below.

AmPure®XP Bead Cleanup

[00216] 1. Vortexing the AmPure® beads until the solution is homogeneous.

2. Adding 40 µl of beads to the products from second strand synthesis and pipetting up and down to homogenize beads.

3. Incubating at room temperature for 10 min.

4. Transferring plate to a magnet and incubating on the magnet for 5 min or until all of the beads have moved towards the magnet.

5. Removing all supernatant.

6. Adding 150 µL of 80% EtOH and incubating for 30 s.

7. Removing supernatant.

8. Repeating steps 6-7.

9. Ensuring that all residual EtOH is removed and removing plate from the magnet and incubating at room temperature for 3 min.

10. Adding 25 µl of low TE buffer and pipetting up and down to homogenize beads.
11. Incubating at room temperature for 2 min.
12. Transferring plate to a magnet and incubating on magnet for 1 min or until all of the beads have move to the magnet.
13. Removing 24 µl of the supernatant and place in a clean plate to store at -20°C.

LabChip QC

For LabChip HS kit

[00217] 1. Removing LabChip and reagents from 4°C to equilibrate to RT (10 mins).
2. Preparing new gel-dye solution if necessary
3. Aspirating and rinsing each of the active chip well with molecular grade H_2O twice.
4. Preparing LabChip ladder by mixing 12 µL ladder solution with 108 µL H_2O in the ladder tube.
5. Preparing 750 µl H_2O in the buffer tube.
6. Using reverse pipetting technique to prepare LabChip.
7. In a BioRad Hardshell or Thermo Fisher Armadillo 96 well plate, diluting 1 µL of library in 19 µL water.
8. Running LabChip.

qPCR Quant

[00218] 1. If needed, preparing qPCR master mix by adding 30 µl Illumina forward and reverse primers to new bottle of Kapa SYBR Fast qPCR MM – 5 mL (KK4601).
2. Preparing 1:10,000 dilution of all libraries.
3. In a BioRad Hard-Shell plate or Thermo Fisher Armadillo plate, preparing the following reaction and leaving at least 12 wells empty.

Reagent	Volume
Kapa SYBR Fast qPCR MM w/ Illumina primers	6 µL
1:10,000 diluted library	4 µL

4. In the same 96 well plate, preparing duplicates of the 6 qPCR standards

Reagent	Volume

Kapa SYBR Fast qPCR MM w/ Illumina primers	6 µL
Library Quantification DNA Standard (1-6 × 2)	4 µL

5. Editing the plate file to reflect plate layout and standard concentrations.

6. Running the following program on the BioRad C1000 thermal cycler: 95°C 5 min > (95°C 30s > 60°C 45s > Image step) × 35.

7. Exporting the qPCR data as an excel sheet.

8. Multiplying starting concentration by (452/300) to adjust for the difference in size between the Kapa standards and the libraries.

9. Multiplying the concentration from step 8 by 10 to adjust for the dilution factor and to convert pM to nM.

Sequencing (NextSeq)

[00219] 1. Thawing 300 cycle NextSeq reagent cartridge by placing in cold water and removing flow cell from 4°C to equilibrate to room temperature.

2. Pooling libraries to be sequenced in a 1:1 molar ratio.

3. Using denature and dilute protocol to dilute library pool to a final concentration of 2.2 pM and a final volume of >1300 µL.

4. Loading 1300 µL in to well 10 of NextSeq reagent cartridge.

5. Emptying the NextSeq waste container and loading a new flow cell and buffer cartridge.

6. Loading the NextSeq reagent cartridge containing libraries in well 10, and settings for NextSeq read length are shown below:

R1	R2	I1	I2
150	150	6	12

7. Sequencing.

CLAIMS

1. A method, comprising ligating a set of adaptors to a library of single-stranded polynucleotides, wherein:

the ligation is catalyzed by a single-stranded DNA (ssDNA) ligase;

each single-stranded polynucleotide is blocked at the 5' end to prevent ligation at the 5' end;

each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide to which the adaptor is ligated, and is blocked at the 3' end to prevent ligation at the 3' end; and

the 5' end of the adaptor is ligated to the 3' end of the single-stranded polynucleotide by the ssDNA ligase to form a linear ligation product,

thereby obtaining a library of linear, single-stranded ligation products.

2. The method of claim 1, further comprising before the ligation step, a step of obtaining the library of single-stranded polynucleotides from a sample.

3. The method of claim 2, wherein the obtaining step comprises denaturing double-stranded polynucleotides from the sample.

4. The method of claim 2 or 3, wherein the sample is a biological sample, optionally wherein: (1) the biological sample is obtained directly from a subject without any treatment; (2) polynucleotides in the biological sample have not been subject to bisulfite conversion; or (3) polynucleotides in the biological sample have been subject to partial or complete bisulfite conversion.

5. The method of claim 4, wherein the biological sample is from a subject having or suspected of having a disease or condition, such as a cancer or neoplasia.

6. The method of claim 5, wherein the biological sample is a sample comprising circulating tumor DNA (ctDNA), such as a blood, serum, plasma, or body fluid sample, or any combination thereof.

7. The method of any one of claims 1-6, wherein the single-stranded polynucleotides are between about 20 and about 400 nucleic acid residues in length.

8. The method of any one of claims 1-7, wherein the ssDNA ligase is a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1, T4 RNA ligase I, thermostable 5' App DNA/RNA ligase, T4 RNA ligase 2, truncated T4 RNA ligase 2, *e.g.*, T4 RNA ligase 2 Truncated, T4 RNA ligase2 Truncated K227Q, T4 RNA ligase2 Truncated KQ, or T4 DNA ligase.

9. The method of any one of claims 1-8, wherein the blocking of each single-stranded polynucleotide comprises dephosphorylation to prevent ligation at its 5' end.

10. The method of any one of claims 1-9, wherein the blocking of each adaptor comprises a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol, and/or hexaethylene glycol, to prevent ligation at its 3' end.

11. The method of any one of claims 1-10, wherein each adaptor comprises a dinucleotide sequence at the 5' end, such as GA (5' to 3'), GG (5' to 3'), AA (5' to 3'), or AG (5' to 3'), which is 5' to the UMI sequence.

12. The method of any one of claims 1-11, wherein the UMI sequence in each adaptor is between about 6 and about 15 nucleic acid residues in length, for example, the UMI sequence is a 12-mer.

13. The method of any of claims 1-12, wherein the ligation reaction is conducted in the presence of a crowding agent.

14. The method of claim 13, wherein the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000 or PEG 6000, Dextran, and/or Ficoll.

15. The method of any of claims 1-14, further comprising converting the library of linear, single-stranded ligation products into a library of linear, double-stranded ligation products.

16. The method of claim 15, wherein the conversion uses a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor.

17. The method of claim 15 or 16, further comprising amplifying and/or purifying the library of linear, double-stranded ligation products.

18. The method of claim 17, wherein the purification selectively purifies polynucleotides between about 50 nucleotides and about 1000 nucleotides in length, the purification being optionally bead-based or column-based, and the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead).

19. The method of any of claims 15-18, further comprising amplifying the library of linear, double-stranded ligation products, *e.g.*, by a polymerase chain reaction (PCR), using:

a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor; and

a primer hybridizable to a target sequence (*e.g.*, an EGFR gene sequence), which primer optionally comprises a sequence selected from the group consisting of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof,

thereby obtaining an amplified library of linear, double-stranded ligation products comprising sequence information of the target sequence.

20. The method of claim 19, wherein a plurality of primers are used, each comprising a sequence specific for the target sequence, wherein the primers have the same or different target sequences, and optionally wherein the plurality of primers comprise any one or more of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

21. The method of claim 19 or 20, wherein the sequence information of the target sequence comprises a mutation, a single nucleotide polymorphism (SNP), a copy number variation (CNV), or an epigenetic change.

22. The method of claim 21, wherein the mutation comprises a point mutation, an insertion, a deletion, an indel, an inversion, a truncation, a fusion, a translocation, an amplification, or any combination thereof.

23. The method of any of claims 19-22, wherein the amplified library of linear, double-stranded ligation products is not a whole genome library.

24. The method of any of claims 19-23, further comprising purifying the amplified library of linear, double-stranded ligation products.

25. The method of claim 24, wherein the purification is bead-based and selectively purifies polynucleotides greater than about 150 nucleotides in lengths, and the purification does not comprise using a specific binding pair (such as biotin/streptavidin), one of which is attached to the linear, double-stranded ligation product and the other is attached to a solid support (such as a bead).

26. The method of claim 24 or 25, further comprising sequencing the purified amplified library of linear, double-stranded ligation products.

27. The method of claim 26, wherein the sequencing step comprises attaching a sequencing adapter and/or a sample-specific barcode to each linear, double-stranded ligation product.

28. The method of claim 27, wherein the attaching step is performed using a polymerase chain reaction (PCR).

29. The method of claim 26, wherein the conversion rate of the sequencing (percentage of single-stranded polynucleotides in the library that give rise to sequencing reads) is at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90%.

30. The method of any one of claims 26-29, which is used for the diagnosis and/or prognosis of a disease or condition in a subject, predicting the responsiveness of a subject to a treatment, identifying a pharmacogenetics marker for the disease/condition or treatment, and/or screening a population for a genetic information.

31. The method of claim 30, wherein the disease or condition is a cancer or neoplasia, and the treatment is a cancer or neoplasia treatment.

32. A library of linear, single-stranded ligation products produced by the method of any of claims 1-14.

33. A library of linear, double-stranded ligation products produced by the method of any of claims 15-18.

34. An amplified library of linear, double-stranded ligation products produced by the method of any of claims 19-25.

35. A sequencing library produced by the method of any of claims 26-31.

36. A kit for constructing a library of ligation products, comprising:

a single-stranded DNA (ssDNA) ligase;

a plurality of adaptors, wherein each adaptor is blocked to prevent ligation at the 3' end while the 5' end of the adaptor is available for ligation to a single-stranded polynucleotide to form a linear, single-stranded ligation product, and each adaptor comprises a unique molecular identifier (UMI) sequence that earmarks the single-stranded polynucleotide.

37. The kit of claim 36, further comprising a denaturing reagent for denaturing a double-stranded polynucleotide from a sample to obtain the single-stranded polynucleotide.

38. The kit of claim 36 or 37, wherein the ssDNA ligase is a *Thermus* bacteriophage RNA ligase such as a bacteriophage TS2126 RNA ligase (*e.g.*, CircLigase™ and CircLigase II™), an archaebacterium RNA ligase such as *Methanobacterium thermoautotrophicum* RNA ligase 1, T4 RNA ligase I, thermostable 5' App DNA/RNA ligase, T4 RNA ligase 2, truncated

T4 RNA ligase 2, *e.g.*, T4 RNA ligase 2 Truncated, T4 RNA ligase2 Truncated K227Q, T4 RNA ligase2 Truncated KQ, or T4 DNA ligase.

39. The kit of any one of claims 36-38, further comprising a dephosphorylating reagent for removing a 5' phosphate group of the single-stranded polynucleotide.

40. The kit of any one of claims 36-39, wherein each adaptor comprises a carbon spacer, ddCTP, ddATP, ddTTP, ddGTP, hexanediol, triethylene glycol, and/or hexaethylene glycol, to prevent ligation at its 3' end.

41. The kit of any one of claims 36-40, wherein each adaptor comprises a dinucleotide sequence at its 5' end, such as GA (5' to 3'), GG (5' to 3'), AA (5' to 3'), or AG (5' to 3').

42. The kit of any one of claims 36-41, wherein the UMI sequence in each adaptor is between about 6 and about 15 nucleic acid residues in length, for example, the UMI sequence is a 12-mer.

43. The kit of any one of claims 36-42, further comprising a crowding agent for the ligation reaction.

44. The kit of claim 13, wherein the crowding agent comprises a polyethylene glycol (PEG), such as PEG 4000 or PEG 6000, Dextran, and/or Ficoll.

45. The kit of any of claims 36-44, further comprising a primer or a set of primers each comprising a sequence that is reverse-complement to the adaptor and/or hybridizable to the adaptor, for converting the single-stranded polynucleotide to a double-stranded polynucleotide.

46. The kit of claim 45, further comprising a reagent for removing primer dimer.

47. The kit of claim 45 or 46, further comprising a primer comprising a sequence specific for a target sequence (*e.g.*, an EGFR gene sequence), for obtaining an amplified linear, double-stranded ligation product comprising sequence information of the target sequence, which primer optionally comprises a sequence selected from the group consisting of SEQ ID NOs: 4-

1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

48. The kit of claim 47, which comprises a plurality of primers, each comprising a sequence specific for the target sequence, wherein the primers have the same or different target sequences, and optionally wherein the plurality of primers comprise any one or more of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

49. The kit of claim 47 or 48, further comprising a sequencing adapter and/or a sample-specific barcode, for sequencing the amplified linear, double-stranded ligation product.

50. The kit of any one of claims 36-49, further comprising separate vials for each component and/or instructions for using the components.

51. The kit of claim 50, wherein the kit further comprises an instruction for obtaining a sample that comprises circulating tumor DNA (ctDNA) from a subject, and/or an instruction for obtaining single-stranded polynucleotides from the sample, such as a blood, serum, plasma, or body fluid sample, or any combination thereof.

52. A polynucleotide, comprising AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 1) or a portion thereof, *e.g.*, a portion that comprises between about 18 and 22 nucleotide residues.

53. The polynucleotide of claim 52, comprising $N_i \ldots N_i$AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG or a portion thereof, wherein N_i to N_i is any nucleic acid residue, for example, A, T, C, or G, and *i* is an integer between about 4 and about 25.

54. The polynucleotide of claim 52 or 53, comprising GANNNNNNNNNNNAGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 2) or a portion thereof, *e.g.*, a portion that comprises between about 32 and 36 nucleotide residues, wherein N is any nucleic acid residue, for example, A, T, C, or G.

55. A polynucleotide, comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof, *e.g.*, a portion that comprises between about 12 and 20 nucleotide residues.

56. A primer, comprising any one or more sequences selected from the group consisting of SEQ ID NOs: 4-1529, or a numerical range or subrange thereof.

57. A primer set, comprising any one or more of SEQ ID NOs: 4-1529, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, or 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof.

58. A primer set, comprising any one or more of SEQ ID NOs: 4-1529, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, or 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof, and a primer comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof.

59. A kit, comprising any one or more SEQ ID NOs: 4-1529, *e.g.*, about 10, 20, 50, 100, 150, 200, 250, or 300, 400, 500, 600, 700, 800, 900, 1,000, 1,100, 1,200, 1,300, 1,400, 1,500, or all of 1529 of SEQ ID NOs: 4-1529, or a complementary or substantially complementary sequence thereof, or a numerical range or subrange thereof, a primer comprising CACTCTTTCCCTACACGACGC (SEQ ID NO: 3) or a portion thereof, and/or a polynucleotide comprising AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTG (SEQ ID NO: 1) or a portion thereof.

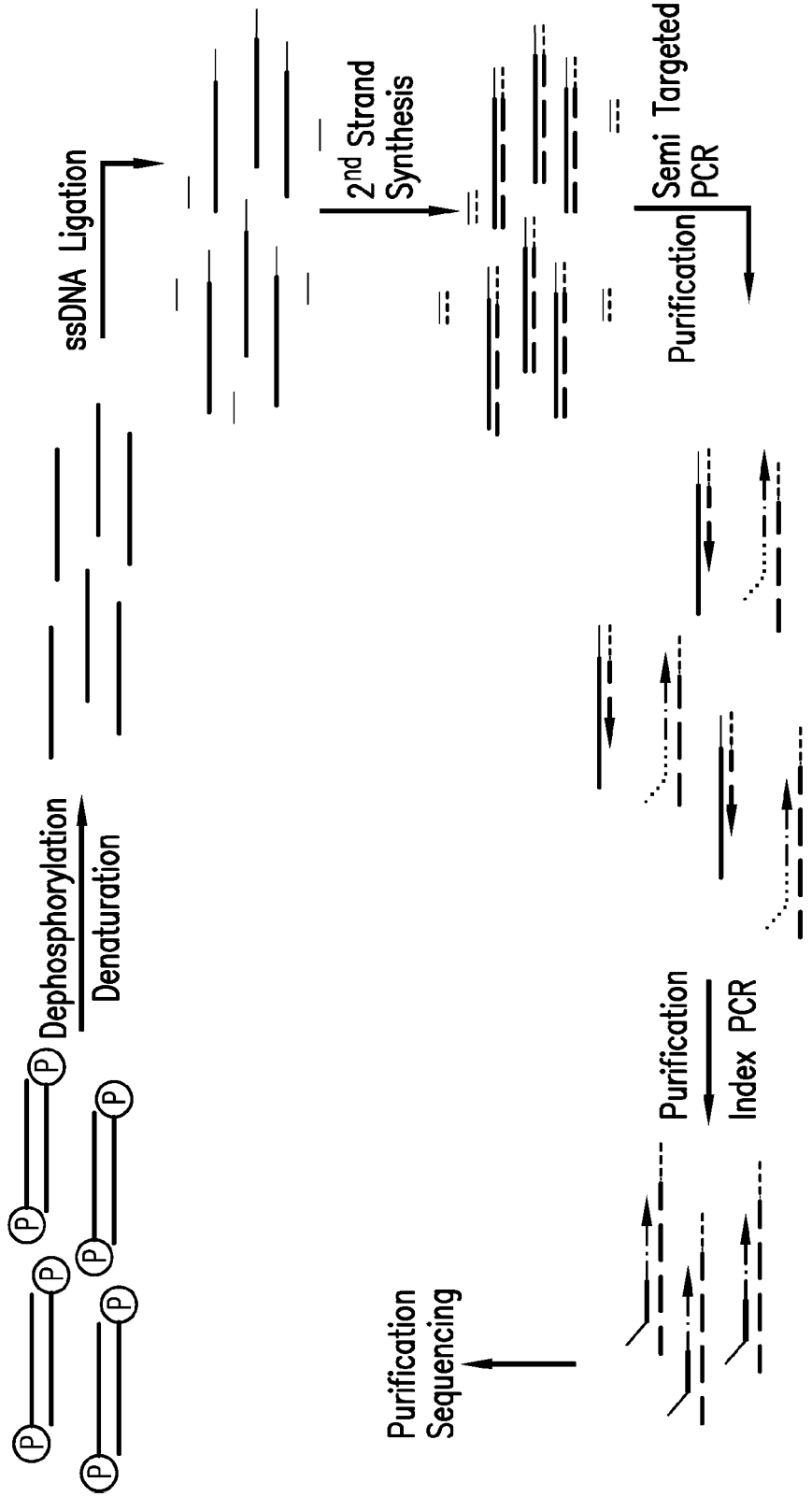

Dephosphorylation
Denaturation
ssDNA Ligation
2nd Strand Synthesis
Semi Targeted PCR
Purification
Purification
Index PCR
Purification
Sequencing

FIG. 1

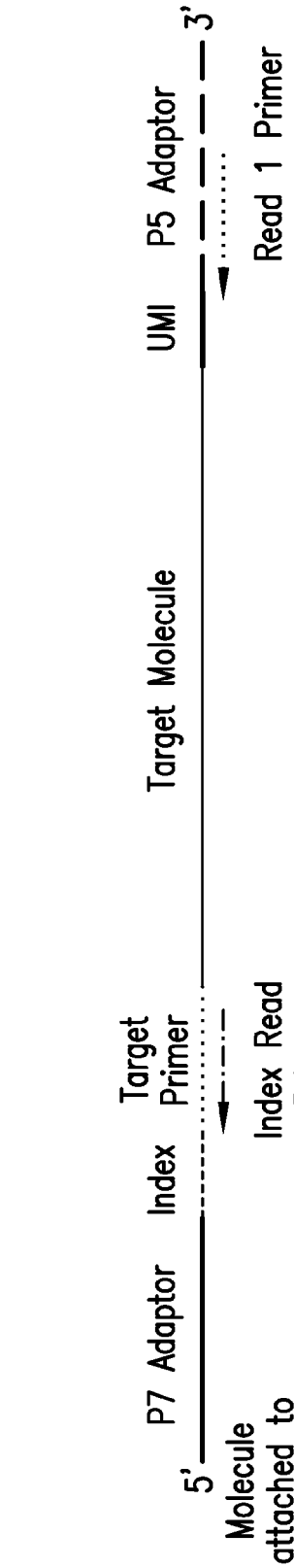

5'
P7 Adaptor
Index
Target Primer
Target Molecule
UMI
P5 Adaptor
3'
Molecule attached to flowcell
Index Read Primer
Read 1 Primer

FIG.2

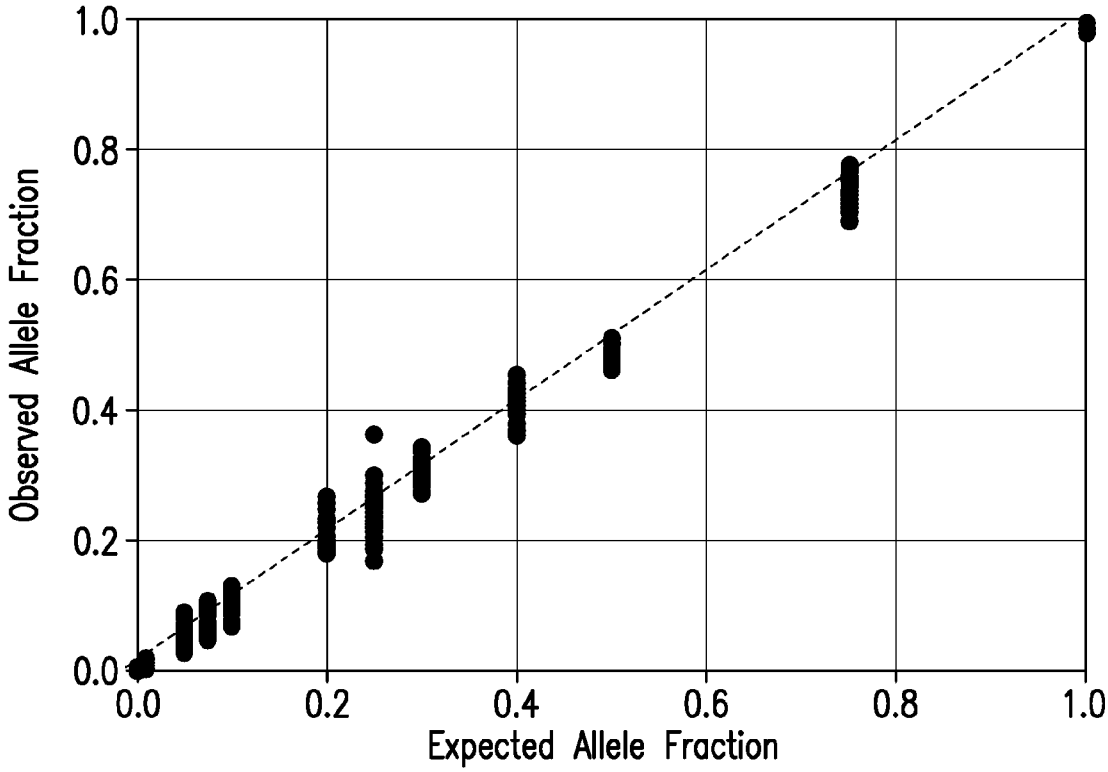

1.0
0.8
0.6
0.4
0.2
0.0
Observed Allele Fraction
0.0
0.2
0.4
0.6
0.8
1.0
Expected Allele Fraction

FIG.3

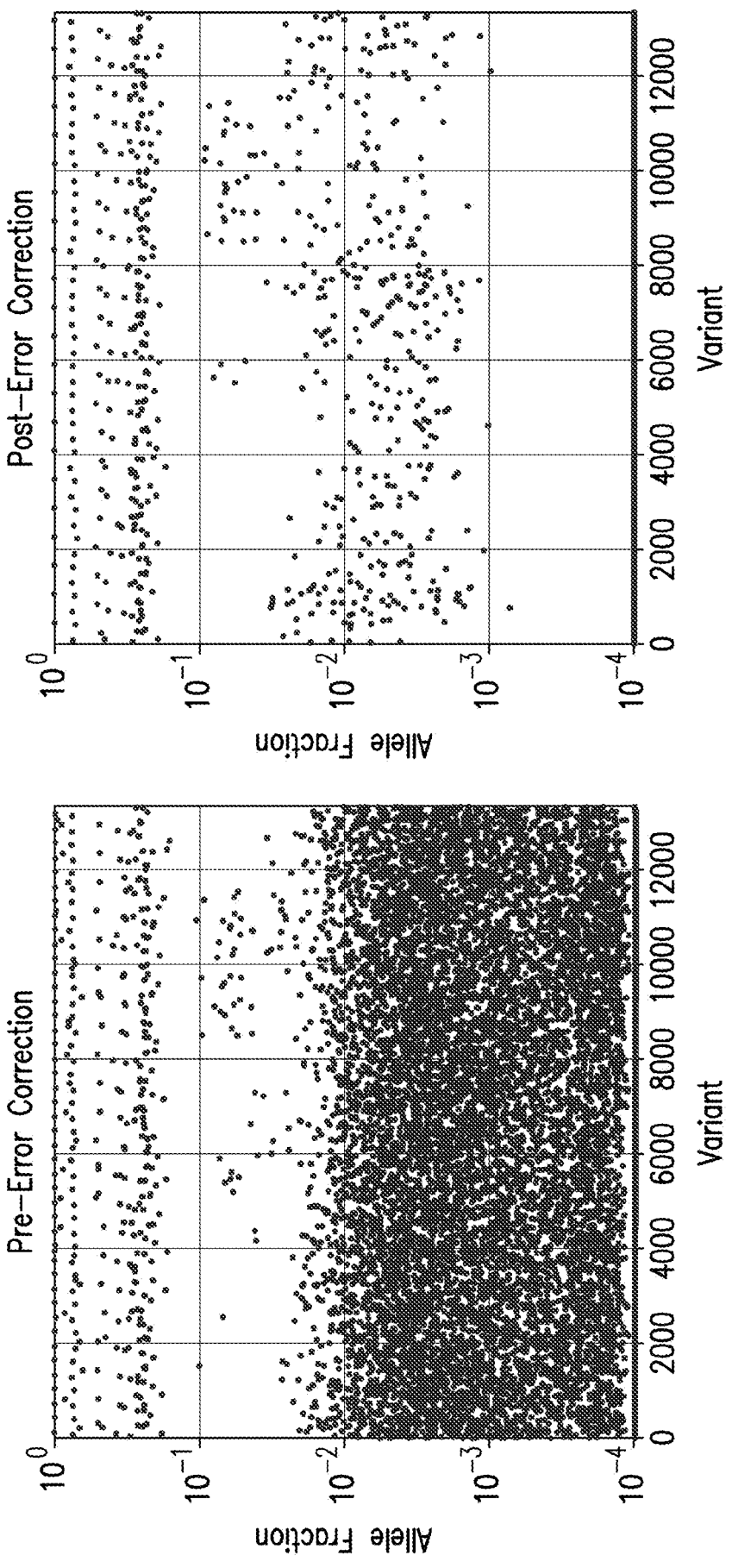

Post−Error Correction
Pre−Error Correction
Allele Fraction
Variant
10^{0}
10^{-1}
10^{-2}
10^{-3}
10^{-4}
0
2000
4000
6000
8000
10000
12000

FIG.4

	The Present Method	Hybridization Capture
Experimental Time	< 1 day	3 days
Minimal MAF	0.1%	0.1%
DNA required	20 ng	30 ng
Ligation efficiency	~90%	~20%
On target rate	>60%	~50%
Uniformity	~0.8	~0.7
Enrichment Factor	>40,000x	~2,000x
Conversion Rate	40–80%	~20%

FIG.5

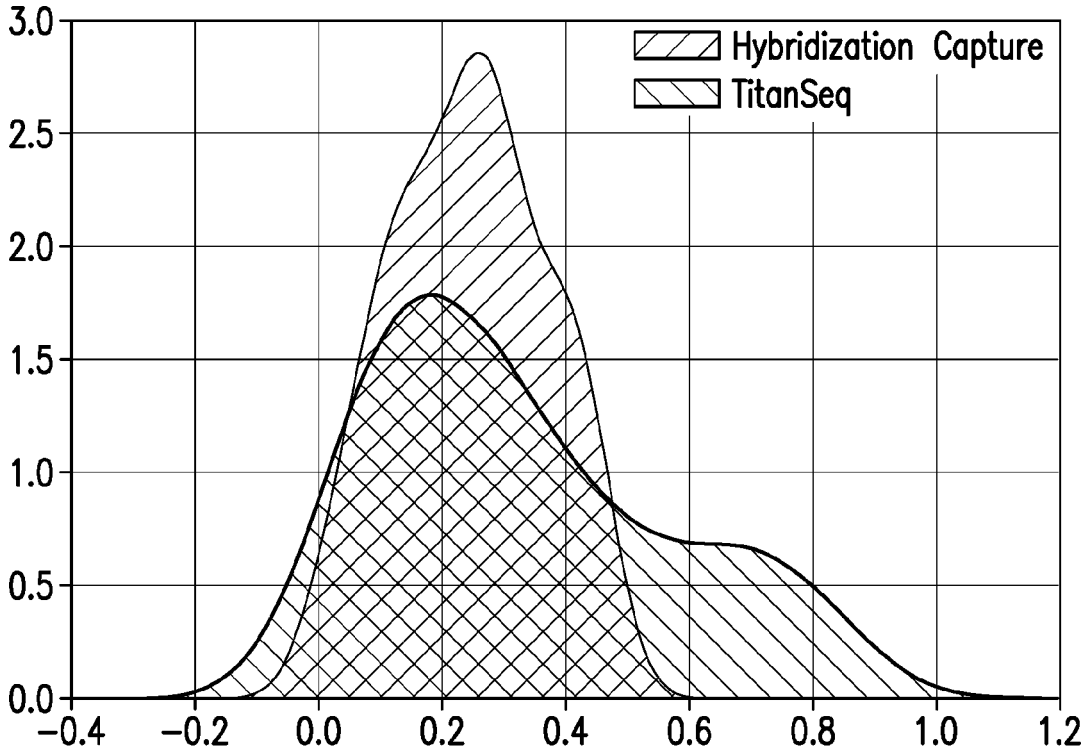

Hybridization Capture
TitanSeq
3.0
2.5
2.0
1.5
1.0
0.5
0.0
−0.4
−0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2

FIG.6

FIG. 7 7/71

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:308	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAAAAGTTCGCC ACCCTTGCCGTTTC
SEQ ID NO:309	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAAATGCATAACA ACAAAGAATATGAATATGGA
SEQ ID NO:310	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACCCAAACTTT ATAAGATCCTGGCT
SEQ ID NO:311	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACCTCTCTTTC TTCCACCTTTCTCC
SEQ ID NO:312	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAACTCTACGTCT CCTCCGACCACTGT
SEQ ID NO:313	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAATACCCCCTCC ATCAACTTCTTCAA
SEQ ID NO:314	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAATCCATCCCCA CACCCTGTTCACTC
SEQ ID NO:315	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACACAGGGCCAA AGACTAAGTGACAT
SEQ ID NO:316	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACACATACAAGT TGGAAATTTCTGGG
SEQ ID NO:317	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACACATCAAGGT TGGAATGAGCTGGA
SEQ ID NO:318	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACATGCCTTTCA CGTTCCTTTCCCC
SEQ ID NO:319	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTCCTTCCCGT TTTTCAGCCACC
SEQ ID NO:320	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTCTACACAGA AAGGGCCCAAATTC
SEQ ID NO:321	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTCTCTTTGAC TGCAGAATCCAACT
SEQ ID NO:322	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTGGAAAAAAC TGTTTGGGACC
SEQ ID NO:323	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAACTGGAAAAAAC TGTTTGGGACCTCC
SEQ ID NO:324	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGAAAGCCCTCC CCAGTCCTCATGTA
SEQ ID NO:325	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGAATCAGAACA ATGCCTCCACGACC
SEQ ID NO:326	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGATCACATCAC ATGAATGGAATAGTTTAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:327	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGATTCAGGCAA TGTTTGTTAGTATTAGT
SEQ ID NO:328	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGATTTACCTCT ATTGTTGGATCATATTCG
SEQ ID NO:329	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCAACCTTTTT TCTCTTTCTCTTTAGA
SEQ ID NO:330	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCACCTGATCC TAGTACCTTCCCTG
SEQ ID NO:331	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCAGCCACACC CCATTCTTGAG
SEQ ID NO:332	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCCAACACACC ACAGATGTCTTCAG
SEQ ID NO:333	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGCCCTCCAACA TCCTAGTCAACTCC
SEQ ID NO:334	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGGACCAAGGAG CAGAGGAGGC
SEQ ID NO:335	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGGGCCTCTTCA TGCGG
SEQ ID NO:336	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTAATTTTGCC CAGTTCAGGATCCA
SEQ ID NO:337	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTAGTCTGATC CACTGAAGCTGAAT
SEQ ID NO:338	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAAGTTCGCCACCC TTGCCGTTTCTTT
SEQ ID NO:339	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATAATAGGAAAT CACAGCTAAGGGGC
SEQ ID NO:340	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATACATTCTTCA TACCAGGACCAGAG
SEQ ID NO:341	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATCACCAAAAAA GTTCGCCACCCTTG
SEQ ID NO:342	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATCAGAACAATG CCTCCACGACCATC
SEQ ID NO:343	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGCATGTTTCC AATTTTAGCGAGTG
SEQ ID NO:344	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATGTAGGAGTGG TCATAAGGCTGGTA
SEQ ID NO:345	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAATTCTACCTTGT AGCCTCCAATGCGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:346	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAAAGTGGTTCT GGATTAGCTGGATT
SEQ ID NO:347	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAAATGGAAAAT ACAACTACGAGAGAAA
SEQ ID NO:348	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAACCCTCCTGC CATCATATTGAACA
SEQ ID NO:349	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAACCTCCGTCA TGTGCTGTGA
SEQ ID NO:350	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAACTACGAGAG AAAAAATGACTTGCT
SEQ ID NO:351	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAACTTAACCTG TTTCTCCTCCCTCT
SEQ ID NO:352	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAACTTACCATG TTCAATGATTTCAACT
SEQ ID NO:353	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAAGGGTATGGG TTTGTCACTGAGA
SEQ ID NO:354	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACAAATGGAAA ATACAACTACGAGAG
SEQ ID NO:355	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACAGTGGAAGA GGTAGATATTGGGG
SEQ ID NO:356	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACATCCCCCAA AGCCAACAAAGAAA
SEQ ID NO:357	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACATTCTTAGA GCATAGTAAGCAGT
SEQ ID NO:358	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACGAGACAAAT GTAGGAAAAAACCA
SEQ ID NO:359	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACTCTAGTATC TGGAAAAATGGCTTTG
SEQ ID NO:360	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACTCTCTGCTG GCTAGTCAAAAAAG
SEQ ID NO:361	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACACTTACCTCAT TGTCTGACTCCACG
SEQ ID NO:362	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGAAAGGACTA TAATGACAGTTAACCC
SEQ ID NO:363	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGAATATTGTT GCTATGGTGATCTTTT
SEQ ID NO:364	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGACTAGCTAG AGACAATGAATTAAGGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:365	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGCAAAACACC AAAAGACCAGACGT
SEQ ID NO:366	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGCACCCTAGA ACCAAATCCAGCAG
SEQ ID NO:367	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGGCGAGGAGT AGCTGTGC
SEQ ID NO:368	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGGGCCAAAGA CTAAGTGACATAAA
SEQ ID NO:369	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGTGAGTGCAG TTGTTTACCATGAT
SEQ ID NO:370	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACAGTGGAAGAGG TAGATATTGGGGAAG
SEQ ID NO:371	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATACAAGTTGG AAATTTCTGGGCCA
SEQ ID NO:372	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATACTTGGACT TGGTGATAGACATGT
SEQ ID NO:373	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATAGAGTTTTA ATGCATTGTCTCATCTT
SEQ ID NO:374	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATAGAGTTTTA ATGCATTGTCTCATCTTTTT
SEQ ID NO:375	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATCAAGTTCAA CAGTTCAGGCCAG
SEQ ID NO:376	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATCGGGGCAAA TTTTTAAAGGCACA
SEQ ID NO:377	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATGCCATCATT CTAGGAAGCTCACC
SEQ ID NO:378	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATTAGGCAGTG ACTCGATGAAGGCA
SEQ ID NO:379	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATTCATAGACA GTAAAACAGAAAGGAC
SEQ ID NO:380	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATTCTTCATAC CAGGACCAGAGGAA
SEQ ID NO:381	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACATTCTTCATAC CAGGACCAGAGGAA
SEQ ID NO:382	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAAATTAAATT ACTCACCTATCTCCCA
SEQ ID NO:383	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAAGCTATATC TGAACAAAAATTCCGT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:384	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCACACCTGTCA TGTAGCAGCTTT
SEQ ID NO:385	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCACATTACATA CTTACCATGCCACT
SEQ ID NO:386	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCACATTACATA CTTACCATGCCACTTTCC
SEQ ID NO:387	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCAGGTAGAGGG AGTACAGAGTGACC
SEQ ID NO:388	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATATACCCAG TGCCTTGTGTGGT
SEQ ID NO:389	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATCTCACAAT TGCCAGTTAACGTCT
SEQ ID NO:390	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATCTCACAAT TGCCAGTTAACGTCT
SEQ ID NO:391	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATCTCACAAT TGCCAGTTAACGTCT
SEQ ID NO:392	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCATGTTCAATG ATTTCAACTAAACTTCT
SEQ ID NO:393	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCCACTGAAAAG CACTTCCTGAAATA
SEQ ID NO:394	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCCTAGCCTTAG ATAAAACTGAGCAAGAGG
SEQ ID NO:395	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCCTTGTCTCTG TGTTCTTGTC
SEQ ID NO:396	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCCTTGTCTCTG TGTTCTTGTC
SEQ ID NO:397	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTAAGCACACA GAGTAATATAGCAG
SEQ ID NO:398	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTAAGCACACA GAGTAATATAGCAGAGC
SEQ ID NO:399	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTAGTATTCTG CTCTGAAGGGGGAAA
SEQ ID NO:400	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTATTGTTG GATCATATTCGTCC
SEQ ID NO:401	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTCTATTGTTG GATCATATTCGTCC
SEQ ID NO:402	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGGAATTTGG ATGTGATTGGAAAGTGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:403	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGTCTTGTCT TTGCTGATGTTTCA
SEQ ID NO:404	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGTCTTGTCT TTGCTGATGTTTCA
SEQ ID NO:405	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTGTCTTGTCT TTGCTGATGTTTCAATA
SEQ ID NO:406	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACCTTCTTTCTAA CCTTTTCTTATGTGCT
SEQ ID NO:407	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGACTGATTCAA AGCTGGTCATTTAGA
SEQ ID NO:408	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGAGACAAATGT AGGAAAAAACCAGAA
SEQ ID NO:409	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGCATTTATGTT TTCTCTTCTTAGACCA
SEQ ID NO:410	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGCATTTATGTT TTCTCTTCTTAGACCATCC
SEQ ID NO:411	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGCTCTTCTCAC TCATATCCTCCTCT
SEQ ID NO:412	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGGCCAGATCCA GTGAAAAACAAGC
SEQ ID NO:413	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGTCTTCCTTCT CTCTCTGTCATAGGG
SEQ ID NO:414	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGTCTTCCTTCT CTCTCTGTCATAGGG
SEQ ID NO:415	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACGTGATTCATTT ATTTGTTCAAAGCAGG
SEQ ID NO:416	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTAAACTTCTAA GATGTGGCAAGATGG
SEQ ID NO:417	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTAAGTAGTCTG ATCCACTGAAGCTG
SEQ ID NO:418	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTACGAGAGAAA AAATGACTTGCTTAA
SEQ ID NO:419	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTAGCTAGAGAC AATGAATTAAGGGAAA
SEQ ID NO:420	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTAGGCGTGGGA TGTTTTTGCAGATG
SEQ ID NO:421	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTATCTCCCTGG GTGTAGCTTTTTAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:422	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTATTCAGTCCT GCCTTCCTGCCC
SEQ ID NO:423	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCAAATGTCTT ACTGCTCTACAAGG
SEQ ID NO:424	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTCTGCTGGC TAGTCAAAAAAGAGA
SEQ ID NO:425	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTCTGGTGGT AGAATGAAAAATAGA
SEQ ID NO:426	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTGTCAAAAA TTGTTTCTGGGGCCA
SEQ ID NO:427	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTGTCCTGCG TCATCATCTTTGTC
SEQ ID NO:428	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTCTTTATTTGT CCCCTTGCCTCCCT
SEQ ID NO:429	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGAAGCCACTT GTTTAATCTGTAGA
SEQ ID NO:430	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGAGCACTGAA TCTATAAAGCATGT
SEQ ID NO:431	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGCCAATGGAC TGTTTTACAATGCC
SEQ ID NO:432	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGTAAAGCTGG AAAGGGACGAACTG
SEQ ID NO:433	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTGTGTTGTGGA GTGCAAGTGAAAGC
SEQ ID NO:434	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTAACCTGTTT CTCCTCCCTCTACC
SEQ ID NO:435	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTACCCACTGA AAAGCACTTCCTGA
SEQ ID NO:436	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTACCTGTCTT GTCTTTGCTGATGT
SEQ ID NO:437	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTACCTGTCTT GTCTTTGCTGATGT
SEQ ID NO:438	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTCCCAGTGTG ATTGCAGGTTC
SEQ ID NO:439	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTCCTGTAATT TTTCAAGGCTTCAG
SEQ ID NO:440	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTCTTTGGGTT GACTTCTCTGGTGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:441	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTGTCCCAAAG CAGAAGTAAAACCA
SEQ ID NO:442	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTACTTTATAAGATC CTGGCTATCCTGTGG
SEQ ID NO:443	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAAAAGTTTGC TGAGCTGGGTAG
SEQ ID NO:444	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAAAGGGGACA TGCTAGGGACAACA
SEQ ID NO:445	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAACACTCTCTG CTGGCTAGTCAAAA
SEQ ID NO:446	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAACACTTACCT CATTGTCTGACTCC
SEQ ID NO:447	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAGCAACATCT CCGAAAGCCAACAA
SEQ ID NO:448	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAGCAAGAAAA TACCCCCTCCATCA
SEQ ID NO:449	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAAGGAGGAGAG ACACCATCAGAAGG
SEQ ID NO:450	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAATGTGTCAGC CTCAAAGAAAAGCT
SEQ ID NO:451	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACACTAAACTC ATCTGGGCCAC
SEQ ID NO:452	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACACTAAACTC ATCTGGGCCAC
SEQ ID NO:453	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACAGAAGGAGG AGAGACACCATCAG
SEQ ID NO:454	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACAGTTGTTTG TTCAGTTGGGAGCG
SEQ ID NO:455	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACAGTTGTTTG TTCAGTTGGGAGCG
SEQ ID NO:456	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACCTAGTATTC TGCTCTGAAGGGGG
SEQ ID NO:457	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACGACTGATTC AAAGCTGGTCATTT
SEQ ID NO:458	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACGACTGATTC AAAGCTGGTCATTT
SEQ ID NO:459	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACTGCTGTGAG GGTTTTTTGATGTT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:460	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGACTTGAAGGCG TATACAGGAACAAT
SEQ ID NO:461	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGAGGGAGTGA AGTGAATGTTGCTG
SEQ ID NO:462	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGATCCCATCC TGCCAAAGTTTGTG
SEQ ID NO:463	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCAATCAGTG AGGAATCAGAGGCC
SEQ ID NO:464	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCAATCAGTG AGGAATCAGAGGCC
SEQ ID NO:465	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCATAGTAAG CAGTAGGGAGTAACA
SEQ ID NO:466	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCCAAGGGTG TGAGTGAACG
SEQ ID NO:467	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGCGTGCAGAT AATGACAAGGAATA
SEQ ID NO:468	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGAGTCATGTTAG TCTGGTTCCTCC
SEQ ID NO:469	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATATTCCCATT ATTATAGAGATGATTGTTGAAT
SEQ ID NO:470	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATGATCCGACA AGTGAGAGACAGGA
SEQ ID NO:471	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGATGTTGAACTA TGCAAAGAGACATTT
SEQ ID NO:472	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAAACTTCTGT ACACAACTAACTAGA
SEQ ID NO:473	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAAGAAAATAC CCCCTCCATCAACT
SEQ ID NO:474	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAAGTATGATG AGCAAGCTTTCTC
SEQ ID NO:475	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCACTACCTAAG GACCGGGATTATGT
SEQ ID NO:476	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCACTCTGACAT ATGGCCATTTCTGT
SEQ ID NO:477	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCAGTAGGGAGT AACAAAATAACACT
SEQ ID NO:478	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCAGTACCTTC CTCTTCTTCTACAT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:479	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCATCCCTGACTGTGAGAT
SEQ ID NO:480	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCATCCCTGACTGTGAGATCAA
SEQ ID NO:481	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCCATCCCTGACTGTGAGATCAA
SEQ ID NO:482	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCTCCAGTTCAGCAAGGGGTCATA
SEQ ID NO:483	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCCTCTTACCTGGAATTTGGATGTGA
SEQ ID NO:484	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCGCTACTAGAAACATGATAGAGGTG
SEQ ID NO:485	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTAAAGGTGAAGATATATTCCTCCAA
SEQ ID NO:486	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTACCTGACCGACGTTGACC
SEQ ID NO:487	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTATATCTGAACAAAAATTCCGTGGT
SEQ ID NO:488	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTGACACCACGATACTTGACAATGA
SEQ ID NO:489	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTTAATAAAAAACCCCGCAGAGAGA
SEQ ID NO:490	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTTAATTCTACCTTGTAGCCTCCAA
SEQ ID NO:491	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTTACCATGGACCCTGACAAATGTG
SEQ ID NO:492	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGCTTCTTCACGCTCCTTCCCTATCC
SEQ ID NO:493	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAAAACTACAATGGAGAAAGAAGACTA
SEQ ID NO:494	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAGATAAGTGATGGAGATGTGATAATTT
SEQ ID NO:495	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGAGTACTTCTTTGGGTTGACTTCTC
SEQ ID NO:496	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCCATCTTCCATCTTCTCACACTGG
SEQ ID NO:497	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCCTTCGTCCTCCTTCCTCACTC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:498	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGCGTGGGATGTTTTTGCAGATGATG
SEQ ID NO:499	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGGAGTGAAGTGAATGTTGCTGAGGT
SEQ ID NO:500	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGGGACATGCTAGGGACAACACGATT
SEQ ID NO:501	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTGTGTCTTTAATTGAAGCATGATTT
SEQ ID NO:502	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTTCACTGCATATTCTCCCCACAGA
SEQ ID NO:503	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGGTTTCATGGACTCAGTTACTACCTG
SEQ ID NO:504	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTACTTACCCACTGAAAAGCACTTCC
SEQ ID NO:505	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCACACTCATCAGCACCAGGTCTTG
SEQ ID NO:506	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCCAACCATGTCAAAATTACAGAC
SEQ ID NO:507	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCCAACCATGTCAAAATTACAGAC
SEQ ID NO:508	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCCCAGCTACTCTCAAAATCAGCA
SEQ ID NO:509	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCCTCCACACTTCTCCATTCTTC
SEQ ID NO:510	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCTATGTGATCAAGAAATCGATAGCA
SEQ ID NO:511	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTCTTTCTTTGAAGCAGCAAGTATGA
SEQ ID NO:512	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGAACGTTGTTGGACTCTACTGTGT
SEQ ID NO:513	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGTGGTATACGATATGGGTTTTGT
SEQ ID NO:514	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGGTTCTGGATTAGCTGGATTGTCA
SEQ ID NO:515	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGTTACTCAAGAAGCAGAAAGGGAA
SEQ ID NO:516	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTGTTACTCAAGAAGCAGAAAGGGAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:517	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTAACTCTCTG GTGGTAGAATGAAA
SEQ ID NO:518	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTCCCTCAGCC GTTACCTGTGT
SEQ ID NO:519	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTGAAAATACA TAGAGTTTTAATGCATTGTC
SEQ ID NO:520	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTGCTAAGAAC CGGTCACTGAAAAT
SEQ ID NO:521	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTAGTTTTTCCTCCT ACTCACCATCCTGT
SEQ ID NO:522	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATAAAAGCTCTTC CTGTTTCAGTCCCC
SEQ ID NO:523	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATACACAAAGAAA GCCCTCCCCAGTCC
SEQ ID NO:524	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATACCCAGTGC CTTGTGTGGTGACT
SEQ ID NO:525	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATGCTCCACTA ACAACCCTCCTGCC
SEQ ID NO:526	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATGGAGAAGTT AGACATGTCAACCT
SEQ ID NO:527	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATGGCCATTTC TGTTTTCCTGTAGC
SEQ ID NO:528	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATTGGCCTGTC TGCTCTTCCCACCA
SEQ ID NO:529	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATATTTGAGTCTA TCGAGTGTGTGCAT
SEQ ID NO:530	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCAAAGACGACT GATTCAAAGCTGG
SEQ ID NO:531	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCAAGTTCAACA GTTCAGGCCAGTGC
SEQ ID NO:532	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCACTCCACATT TCAGCAACAGCAGC
SEQ ID NO:533	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCATATTGGCCT GTCTGCTCTTCCCA
SEQ ID NO:534	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCCACTCAATCT TCTACTTTAAAATGACTT
SEQ ID NO:535	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCGCGGGCTTGG TTCTGATGTTTGTA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:536	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTCCCTTCTAC CGGCAGATCCC
SEQ ID NO:537	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTCCCTTCTAC CGGCAGATCCCTTT
SEQ ID NO:538	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTGATGCTGAG GAAGTGGATTTTGC
SEQ ID NO:539	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTGGAGATCAA ACCCGCAATCCG
SEQ ID NO:540	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATCTGGAGCATGG GACTGTCTCTGGTA
SEQ ID NO:541	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGAACATGACCC TGAATTCGGATGCA
SEQ ID NO:542	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGACTGAGACAA TAATTATTAAAAGGTGATCT
SEQ ID NO:543	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGAGTTCTGGGC ACTGGGTCAAAGTC
SEQ ID NO:544	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCAGAGCTTCT TCCCATGATGATC
SEQ ID NO:545	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCGCTTGACAT CAGTTTGCCAGTTG
SEQ ID NO:546	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCGCTTGACAT CAGTTTGCCAGTTG
SEQ ID NO:547	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGCTCTGCTTCT GTACTGCCAG
SEQ ID NO:548	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGAACTGATGT CTGGACGCTCATTG
SEQ ID NO:549	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGAAGAAATCG GTAAGAGGTGGGCC
SEQ ID NO:550	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGAAGGTGCGT TCGATGACAGTG
SEQ ID NO:551	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGACATGAAGC AGGCTGATAC
SEQ ID NO:552	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGACATGAAGC AGGCTGATACTAC
SEQ ID NO:553	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGACCATTTAA CACAGAAGAGAGT
SEQ ID NO:554	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGCAGTCAAAC CTTCTCTCTTATGT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:555	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGCTGTGGTTTGTGATGGTTGGGAG
SEQ ID NO:556	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGGAGAACTCTGAGTGGCCACCTC
SEQ ID NO:557	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGGTGAATGACGGCGTGGAGGAC
SEQ ID NO:558	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGTATTGGTCTCTCATGGCACTGTA
SEQ ID NO:559	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGTCTTACTGCTCTACAAGGGCTTTA
SEQ ID NO:560	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATGTTATTTGAGCTAGAACCAGTGCCA
SEQ ID NO:561	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTACATGTGGAGTGAACGTTGTTGGA
SEQ ID NO:562	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTAGTGATGGATTTGATGAATTGGTGAT
SEQ ID NO:563	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTCTGCTCTGAAGGGGGAAATGTGAG
SEQ ID NO:564	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTCTGTGTGTAAAGCCCAGCCCCC
SEQ ID NO:565	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTGAAATTCACTTACACCGGGCCCTC
SEQ ID NO:566	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTGTGAAGATCTGTGACTTTGGCCTG
SEQ ID NO:567	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTTATTTCAGTGTTACTTACCTGTCTTGT
SEQ ID NO:568	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTTCAACTAAACTTCTAAGATGTGGCA
SEQ ID NO:569	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTTGAGGGGGAGTCTGGGAATGAACA
SEQ ID NO:570	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTATTTTTTATGGCAGTCAAACCTTCTCT
SEQ ID NO:571	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAACGCAGCCCAGGACGAGTATG
SEQ ID NO:572	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAACCAGACCTCAGGCGGCTCATAG
SEQ ID NO:573	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAACTAGCCCTCAATCCCTGACCCTG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:574	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAACTAGCCCTC AATCCCTGACCCTG
SEQ ID NO:575	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAGACGACTGA TTCAAAGCTGGTCA
SEQ ID NO:576	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAGCAGCCTCT CTTAACCCCCTTCC
SEQ ID NO:577	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAGTCTGTGGC CTTGTACTGCAGA
SEQ ID NO:578	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAAGTTTGGGAA GGCTGGAAGGGAC
SEQ ID NO:579	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAATGTCTTACT GCTCTACAAGGGCT
SEQ ID NO:580	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAATTGTTGCCA TTTCAGGGTTTCT
SEQ ID NO:581	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACACACCACAG ATGTCTTCAGGCTT
SEQ ID NO:582	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACCAGCCCTGT CGTCTCTCCAG
SEQ ID NO:583	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACCCAATAGAC CCACCCCAATCTCC
SEQ ID NO:584	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACGGGTTCCTT CCTTCGAGAGCTTC
SEQ ID NO:585	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAACTTGGAGGCC TTGCAGAAGAA
SEQ ID NO:586	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGAGATCTGAT GCTGAGGAAGTGGA
SEQ ID NO:587	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGCCTCACACC ACCCCCAC
SEQ ID NO:588	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGAGATAAGT GATGGAGATGTGATAA
SEQ ID NO:589	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGGGCTAGGA TGGGGACTCTTG
SEQ ID NO:590	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGGTGTGAGT GAACGGTGAGC
SEQ ID NO:591	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAAGGTGGAAAGA GGAAGATGAGAAC
SEQ ID NO:592	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAATAGACCCACC CCAATCTCCCCAGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:593	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAATCAAACTGCA GAGTATTTGGGCGA
SEQ ID NO:594	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAATCCTCGGCCT CTAGTGTGCAGA
SEQ ID NO:595	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAAAATGGTTC TGGATCAGCTGGA
SEQ ID NO:596	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAAGCCACCCA TCTCCTCAGCTG
SEQ ID NO:597	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAAGGGGCTAG GATGGGGACTC
SEQ ID NO:598	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACACCACCCCCA CCCACAGATC
SEQ ID NO:599	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGAGAAGTTG TTGAGGGGAGCCT
SEQ ID NO:600	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGATGTTCCC GGGGCTGC
SEQ ID NO:601	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGCCCACGTA CCGCTCCTC
SEQ ID NO:602	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACAGCTGGAAGG ACAAGCC
SEQ ID NO:603	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCAGCCGCGAA ACCTGAGAAG
SEQ ID NO:604	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCAGGACATGC ACAGCTACATCG
SEQ ID NO:605	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCCAAATGTGC AGAAAGACCT
SEQ ID NO:606	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCCCCACCCAC AGATCCACT
SEQ ID NO:607	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCCCTTCCCCA GTGCATCCA
SEQ ID NO:608	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCCTGCCATGC TACCTAGATACCTT
SEQ ID NO:609	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCCTGTTCACT CCTTTGCTGATTGG
SEQ ID NO:610	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCGCTTCTTGT CCTGCTTGCTTAC
SEQ ID NO:611	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACCTGATCCTAG TACCTTCCCTGCAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:612	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACGCTCCATTAT CCAGCCCCAAAG
SEQ ID NO:613	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACGTGTTGAAGT CCTCGTTGTCTTGT
SEQ ID NO:614	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACGTGTTGAAGT CCTCGTTGTCTTGT
SEQ ID NO:615	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCAGATCTCG TCAGCCATGGAGT
SEQ ID NO:616	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTCGGATAAGA TGCTGAGGAGGGG
SEQ ID NO:617	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGACCCACCA CCCCCTCAC
SEQ ID NO:618	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGCCGCTTCC CCACCAG
SEQ ID NO:619	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGCCTCATCT CTCACCATCCCAAG
SEQ ID NO:620	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTGCTACCACA AGTTTGCCCACAA
SEQ ID NO:621	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACTTTGACTCAC CGGTGGATGAAGTG
SEQ ID NO:622	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAAAAGCGGCT GTTAGTCACTGG
SEQ ID NO:623	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAAGATGACAG GGGCCAGGAG
SEQ ID NO:624	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACATTGTGCA AGCAGGTCCCTC
SEQ ID NO:625	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACGCATTTCC ACAGCTACACCATA
SEQ ID NO:626	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGACTGATGGCC AACTCCCCTTC
SEQ ID NO:627	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAGAGCAACAA ACCACTGGAAT
SEQ ID NO:628	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAGAGCAACAA ACCACTGGAATATA
SEQ ID NO:629	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAGCCCAGTCC CCCTCAGG
SEQ ID NO:630	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAGGGGAGTTG GGGTGAGGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:631	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGAGTAGGGGCT GGCTGGATGAG
SEQ ID NO:632	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGATCTGTATTT ATTTCAGTGTTACTTACCT
SEQ ID NO:633	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGATGCTTTGGA ATGAGTGTTAGAAC
SEQ ID NO:634	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCATTGTTGGG GGACACGAGC
SEQ ID NO:635	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCACACATGC CATCATTCTAGGA
SEQ ID NO:636	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCACGGGTAA TAATTTTTGTCCTT
SEQ ID NO:637	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCGATGTCAG TCTGGTGTGG
SEQ ID NO:638	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCGGTTCTCT GCACATTGGAA
SEQ ID NO:639	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCTACAGAGT CCGCAAGCCAAG
SEQ ID NO:640	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGCCTCTCTTAA CCCCCTTCCCTAG
SEQ ID NO:641	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCACAGCTTT TCCTCCATGAG
SEQ ID NO:642	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCACAGCTTT TCCTCCATGAGTAC
SEQ ID NO:643	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCATCCTCAG CTACGGGGT
SEQ ID NO:644	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCCCTGGTAG CTCATCATC
SEQ ID NO:645	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGCCTCTCTGT CTGAACTTGGG
SEQ ID NO:646	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGGAGCAGCGA GGCCTTCAC
SEQ ID NO:647	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGGCATGAACT ACTTGGAGGACC
SEQ ID NO:648	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGGGTACTTAG ATGGGGGATGGCTG
SEQ ID NO:649	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTCTCCGTGG ATGCCTTCAAG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:650	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTTCCACACA CAGGCGTCC
SEQ ID NO:651	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGGTTCTTTGGG GGCAGAGGG
SEQ ID NO:652	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTATAGAGCGT GCAGATAATGACAA
SEQ ID NO:653	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTCGCCTCAGT AAAGCCACCTCAC
SEQ ID NO:654	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTGGGGCAGAC TCTCTCCTCC
SEQ ID NO:655	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTGGGGCAGAC TCTCTCCTCC
SEQ ID NO:656	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTGTTTGTTC AGTTGGGAGCGGAG
SEQ ID NO:657	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCAGTTGTTTGTTC AGTTGGGAGCGGAG
SEQ ID NO:658	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATACGTCTTGGT TCACTCATCCGGGA
SEQ ID NO:659	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCAGCACCACC ACCACCAGC
SEQ ID NO:660	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCCACTGCTAC CACAAGTTTGCCC
SEQ ID NO:661	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCCAGTGCCAG AACCCGCTCTTC
SEQ ID NO:662	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCGTCTTTGCA GGCCTCTCT
SEQ ID NO:663	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCTGCCTCACC TCCACCGTG
SEQ ID NO:664	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATCTTCCATCTT CTCACACTGGGGGT
SEQ ID NO:665	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGAACTCCACA TTTGCCTTGGGACC
SEQ ID NO:666	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGCCTGGCTCC CTAATTTTATAGTT
SEQ ID NO:667	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGCGCTTGACA TCAGTTTGCCAG
SEQ ID NO:668	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGCTGCAGGGA GGGGGC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:669	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGGAACCAGAC AGAAAAGCGGCT
SEQ ID NO:670	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGGACCCTGAC AAATGTGCTGTTCT
SEQ ID NO:671	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATGTCTGGCACT GCTTTCCA
SEQ ID NO:672	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTCTTGAGGGG CTGAGGTGGAAGAG
SEQ ID NO:673	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTGGCATGGGG AAATATAAACTTGT
SEQ ID NO:674	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTGGGGAGGTA GAGGGCACAC
SEQ ID NO:675	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTGTTGGGGGA CACGAGCCTG
SEQ ID NO:676	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTTCCATCTCC CCTCCCTTTACCCT
SEQ ID NO:677	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTTCTTCCTTT TCCATGCAGTGTGT
SEQ ID NO:678	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCATTTGGATGCCT TATTGCGACAGATC
SEQ ID NO:679	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAAAGCAGCCTC TCTTAACCCCCT
SEQ ID NO:680	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAATGGAAAAGA AATGCTGCAGAAACA
SEQ ID NO:681	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAATTCAGGACC CACACGACGG
SEQ ID NO:682	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACACACCACCC CTCTGCTGG
SEQ ID NO:683	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACACTTCTCCA TTCTTCACAAGGGTATG
SEQ ID NO:684	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACATTTCAGCA ACAGCAGCATCTATA
SEQ ID NO:685	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACATTTCAGCA ACAGCAGCATCTATAAGA
SEQ ID NO:686	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACCAAGCAGCC CATCCCTG
SEQ ID NO:687	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACGATGCCCAG TCAATCTTGTGTAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:688	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACGATGCCCAG TCAATCTTGTGTAATTT
SEQ ID NO:689	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTAACAACCC TCCTGCCATCATATT
SEQ ID NO:690	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTCAATCTTC TACTTTAAAATGACTTAGG
SEQ ID NO:691	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTCAATCTTC TACTTTAAAATGACTTAGGAAA
SEQ ID NO:692	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTCATGCTCT ACAACCCCACCAC
SEQ ID NO:693	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTCTTGCTCC TTCCATCCTTGCTC
SEQ ID NO:694	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTGACAACCA CCCTTAACCCCTC
SEQ ID NO:695	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTGCTGGCTG ATCTATGTCCCTG
SEQ ID NO:696	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCACTTCTACGAC TTCTTCAACCAGGC
SEQ ID NO:697	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAAGGTCTAC ATGGGTGCT
SEQ ID NO:698	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAAGGTCTAC ATGGGTGCTTCC
SEQ ID NO:699	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAAGGTCTAC ATGGGTGCTTCC
SEQ ID NO:700	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGAAGGTCTAC ATGGGTGCTTCC
SEQ ID NO:701	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGACCTAACTC TTGAATGACCCTGT
SEQ ID NO:702	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGACCTAAGAG CAATCAGTGAGGAATCA
SEQ ID NO:703	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCCAGACCCA GCCAGTATTATTTC
SEQ ID NO:704	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCGTGTCCTC TCTCCTCCATAG
SEQ ID NO:705	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCGTGTCCTC TCTCCTCCATAG
SEQ ID NO:706	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCTGCTCACC ATCGCTATCTGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:707	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCTGCTCACC ATCGCTATCTGA
SEQ ID NO:708	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGCTGGGTGAA CTTTGAGGCC
SEQ ID NO:709	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGAGCTAATA AAAATAACTTCTTTCTCTGG
SEQ ID NO:710	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGGAGCAGCG AGGCCTT
SEQ ID NO:711	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGGAGGGAGG CCAGCTG
SEQ ID NO:712	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGGGTCCAAG TTAGGTTAGGTGAT
SEQ ID NO:713	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGGTCCACGGG CAGAC
SEQ ID NO:714	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTGTTTCTTT TAAATACCTGTTAAGTTTGT
SEQ ID NO:715	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCAGTTGGTTACA TACTTGGACTTGGTGAT
SEQ ID NO:716	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATAAAGACAGA AGGAGGAGAGACACCA
SEQ ID NO:717	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATAAAGGCTTT AACACAGAATCAAAAG
SEQ ID NO:718	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCATGCTGAG GTGCCACA
SEQ ID NO:719	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCCCAAGGTG CCTATCAAGTGGA
SEQ ID NO:720	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCCCCAGTGA CTGTGTGTTGATCA
SEQ ID NO:721	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCCCCGTGTC CCTCCTAAGC
SEQ ID NO:722	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCTCCCCTCC CTTTACCCTTTCTT
SEQ ID NO:723	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCTGTCCTGG GCATGTCTCTG
SEQ ID NO:724	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATCTTCTCTTT AGGGTCGGATTCCA
SEQ ID NO:725	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGAGTTCTGG GCACTGGGTCAAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:726	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATGGGAGAACT CTGAGTGGCCAC
SEQ ID NO:727	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTATCTTCAG CTTTCTCCCACTGT
SEQ ID NO:728	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTCCAGGGGA TGAGCTACCT
SEQ ID NO:729	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTCTTGAGGG GCTGAGGTGGAA
SEQ ID NO:730	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTTTAGACCT TGAGTTCTTGAGTTC
SEQ ID NO:731	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTTTGAAAGT GCCGGCCCG
SEQ ID NO:732	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAAACTAGCCC TCAATCCCTGACC
SEQ ID NO:733	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAAATATTCTC CAGGCGTTTCTTCCA
SEQ ID NO:734	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAATCTACCTG TGTCAGTTCCCTCC
SEQ ID NO:735	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCACAGATGTTC CCGGGGC
SEQ ID NO:736	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCACCACAGCTA GAACTTATCAAACCC
SEQ ID NO:737	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCACGCTCTTCT CACTCATATCCTCC
SEQ ID NO:738	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCACGCTCTTCT CACTCATATCCTCC
SEQ ID NO:739	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCACGTACCGCT CCTCAGGA
SEQ ID NO:740	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGAACTAACA GGTTAAGTGCTCCCA
SEQ ID NO:741	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGAGCCCAGT CCCCCTC
SEQ ID NO:742	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCAGCTGCTCAC CATCGCTATC
SEQ ID NO:743	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATCCCCAGTG ACTGTGTGTTGA
SEQ ID NO:744	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATTATTATAG AGATGATTGTTGAATTTTCC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:745	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCATTATTATAG AGATGATTGTTGAATTTTCCTTT
SEQ ID NO:746	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCCAGCTACTCT CAAAATCAGCATCC
SEQ ID NO:747	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCCATTAAATGA GGTTTTACTGTTGT
SEQ ID NO:748	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCGTTCCATCAT AGCATGCAAGGG
SEQ ID NO:749	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTAATCACCAC CCCACCCAATTCC
SEQ ID NO:750	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTATCCTGGCT GTGTCCTG
SEQ ID NO:751	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTCCCCAGCTG CCTTCCA
SEQ ID NO:752	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTCCCCTCGAA ATGAAGCTACAACA
SEQ ID NO:753	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTCGGGTCCCT GCTCTG
SEQ ID NO:754	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGACAAATGT GCTGTTCTTCTTGGT
SEQ ID NO:755	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGATAGTTGC TAAGAACCGGTCAC
SEQ ID NO:756	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGCAAAGACA AATGGTGAGTACGT
SEQ ID NO:757	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTGTTCACTCC TTTGCTGATTGGTTT
SEQ ID NO:758	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTTGCCTCCCT TTCCAATGGACTAT
SEQ ID NO:759	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCCTTTGAACTTG CTCCCTCAGGCTAC
SEQ ID NO:760	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGAAGTGTAAGC CCAACTACAGAAATGG
SEQ ID NO:761	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGACAAGTGAGA GACAGGATCAGGTC
SEQ ID NO:762	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGCAGTTCCATT CTCCCGCAG
SEQ ID NO:763	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGGGGATTAAA GCTGGCTATGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:764	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGTAGTTGCCCTTCTCGAACATGT
SEQ ID NO:765	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGGTGTAGGAGCTGCTGGTG
SEQ ID NO:766	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCGTGTCCCTCCTAAGCGCTGG
SEQ ID NO:767	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTACACTGCACCCCTCTCCTCC
SEQ ID NO:768	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTAGACTTGGGTGAGGCAGGG
SEQ ID NO:769	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTATAGCTCCTGAGTATTGGTGTTCC
SEQ ID NO:770	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTATCTCCCAGGCCTAAAATATACCCA
SEQ ID NO:771	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCACAGCAGGGTCTTCTCTG
SEQ ID NO:772	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCACCTGTCTACGTTCCCTCACT
SEQ ID NO:773	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCAGGATCCATTTCTGCCCAGTG
SEQ ID NO:774	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCCAGTTCAGCAAGGGGTCATAGAC
SEQ ID NO:775	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCCCCGGTGCGCATGTACT
SEQ ID NO:776	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCCCTGGTCAGAGTTCAAGTA
SEQ ID NO:777	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCGGGTCCCTGCTCTGTCA
SEQ ID NO:778	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTCTGTCTGAACTTGGGCAA
SEQ ID NO:779	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTCTTTCTTCCACCTTTCTCCAGC
SEQ ID NO:780	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTCTTTCTTCCACCTTTCTCCAGC
SEQ ID NO:781	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTGGCATTCTGGGAGCTTCATC
SEQ ID NO:782	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTCTGGTCAAGGTCACATTCTT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:783	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGAGCCTGTTTTGTGTCTACTGTTCT
SEQ ID NO:784	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGAGTCATTTCTTCCTTTTCCATGCA
SEQ ID NO:785	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGAAGTTTAGGTCAAAGAGGCTGC
SEQ ID NO:786	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGATCATGGCAGGCTTTGG
SEQ ID NO:787	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGCCTTCTCCTTTACCCCTCC
SEQ ID NO:788	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGGGTGACGGATGCC
SEQ ID NO:789	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGTAGCTCATCATCTGGGACA
SEQ ID NO:790	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGTAGGTTTTCTGGGAAGGGACA
SEQ ID NO:791	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGGTATGGTCATGGAAGGGG
SEQ ID NO:792	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTGCTTCAACTAAATTTAACTGTCA
SEQ ID NO:793	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTGGATTTTTAGGCCCTTGTATTTGT
SEQ ID NO:794	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTGTGTGTGGTGATATCAAAGTAGAGT
SEQ ID NO:795	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTAGTCTTTCTTTGAAGCAGCAAGT
SEQ ID NO:796	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTATTACTTGGGAGACTTGTCTGAACA
SEQ ID NO:797	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCCCTGCAAAGACAAATGGTGAGT
SEQ ID NO:798	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCGCCTGTCCTCATGTATTGGTCT
SEQ ID NO:799	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTCTAGTAATTTGGGAATGCCTGGT
SEQ ID NO:800	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTGGGTATTTTTATGGGAGGCAGAA
SEQ ID NO:801	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTGGTGACCGCTCTGCATCTAGTG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:802	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTCCGAATGC CAAACACCTTCATG
SEQ ID NO:803	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTCTGTAGGC TGGATGAAAAATTC
SEQ ID NO:804	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTCTGTAGGC TGGATGAAAAATTC
SEQ ID NO:805	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTCTTTGCAG GGGTGGCTATGTAG
SEQ ID NO:806	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCTTTGTCCCTCC CACCCCAAACTAG
SEQ ID NO:807	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAAAGACCCTAG CCTTAGATAAAACT
SEQ ID NO:808	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAAATGAAGCTA CAACATCACCACGG
SEQ ID NO:809	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAATGAGGGTGA TGTTTTTCCGCGG
SEQ ID NO:810	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAATGCAGTTTT TCCTCCTACTCACCA
SEQ ID NO:811	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGACTCGTGCTAT TTTTCCTCACAGCT
SEQ ID NO:812	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGACTTTGTGACC TTCGGCTTTTTCAA
SEQ ID NO:813	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAGGGGGGCGTC AGGAAC
SEQ ID NO:814	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGAGTGAGCTGCG AGACCTGC
SEQ ID NO:815	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGATCTGGGACTG CATGCTGGTG
SEQ ID NO:816	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCAGGAAGTGGA AGGAGCTGTTG
SEQ ID NO:817	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCAGTGCTAACC AAGTTCTTTC
SEQ ID NO:818	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCAGTGCTAACC AAGTTCTTTCTTT
SEQ ID NO:819	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCCCATGTCTTT GCAGCCGAG
SEQ ID NO:820	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCCGGTAGTTGC CCTTC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:821	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCCGGTAGTTGCCCTTCTCG
SEQ ID NO:822	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCTGGTTTGGGTGCTGTGTCC
SEQ ID NO:823	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGCTTCTTGTCCTGCTTGCTTACCTC
SEQ ID NO:824	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGAAGCAACCCACAGATGTTCC
SEQ ID NO:825	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGACATCAGCAAAGACCTGGAGAAG
SEQ ID NO:826	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGCTGCTGGACATTGACGAGAC
SEQ ID NO:827	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGAGCGCGAGGAGGAGC
SEQ ID NO:828	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGATGGGGCCACACTTACTCTG
SEQ ID NO:829	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTCATCACCACGCTCCATTA
SEQ ID NO:830	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGCTTGGTTCTGATGTTTGTAGTGT
SEQ ID NO:831	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGTATGGCAGCAGGTATATCTCAGG
SEQ ID NO:832	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGGGTCTCTCGGAGGAAGGACTTGA
SEQ ID NO:833	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCAGCCTGAACATAACATCCTTGGG
SEQ ID NO:834	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCCACAAAATGATTCTGAATTAGCTGT
SEQ ID NO:835	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCCTCTCGTTTCCTTACATGCAGG
SEQ ID NO:836	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCGTGGAGAACAAGTTTGGCAGC
SEQ ID NO:837	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCTCCTCCGGCCCCTCG
SEQ ID NO:838	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTCTCTGGATGGAACTGATGTCTGG
SEQ ID NO:839	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCGTTCTTTTCCACGTGCTTGATCCACT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:840	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAAGTGCTGGGATTACTGGTGTGAGC
SEQ ID NO:841	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTACACTGCACCCCTCTCCTCCCAG
SEQ ID NO:842	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTAGACTTGGGTGAGGCAGGGGTG
SEQ ID NO:843	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTATGTGCTCAGTTCCCTCCTCTATGC
SEQ ID NO:844	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAAAAGCCTCCAGTCGCCTCAGTA
SEQ ID NO:845	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAACGCCCATGTCTTTGCAGCC
SEQ ID NO:846	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAACGCCCATGTCTTTGCAGCC
SEQ ID NO:847	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCACACCACCCCCACCCACAG
SEQ ID NO:848	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCACCGCAGTTCCATTCTCCCG
SEQ ID NO:849	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCACCTCCACCGTGCAGCTC
SEQ ID NO:850	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCACCTGGGGCCACATTTGAACATTG
SEQ ID NO:851	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCACCTGTCTACGTTCCCTCACTGTA
SEQ ID NO:852	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAGATCTCGTCAGCCATGGAGTACC
SEQ ID NO:853	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCAGGGGGCAGCATTGTTGGG
SEQ ID NO:854	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCATCACCACGCTCCATTATCCAGC
SEQ ID NO:855	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCATGCTCTACAACCCCACCACGTAC
SEQ ID NO:856	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCACTAACAACCCTCCTGCCATCAT
SEQ ID NO:857	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCACTTTTGCACAGCCAAGAACACT
SEQ ID NO:858	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCAGTCTCCCTCCTGTTTGCACA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:859	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCCACCCCAAA CTAGCCCTCAATC
SEQ ID NO:860	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCCAGGCCTAA AATATACCCAACCA
SEQ ID NO:861	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCCATACCCTC TCAGCGTACCCTTG
SEQ ID NO:862	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCCCGAGTGAG CTGCGAGAC
SEQ ID NO:863	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCTCCACCTCC TCCTCCATTGG
SEQ ID NO:864	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCCTTCCATCCT TGCTCCTGTCCTTG
SEQ ID NO:865	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGGGTCCCTGC TCTGTCACTG
SEQ ID NO:866	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCGTGTTGTCTC TCCTCCTGTCAGTG
SEQ ID NO:867	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTCTCCTCCCC ACTGCTGCTG
SEQ ID NO:868	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTCTGTTTTAA GATCTGGGCAGTGA
SEQ ID NO:869	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTCTTAACCCC CTTCCCTAGCTGTG
SEQ ID NO:870	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGCCTCAATA AGCCAACCATGTCT
SEQ ID NO:871	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGCTACTGG TGATGCTGTCC
SEQ ID NO:872	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGGCTACTGG TGATGCTGTCCAAG
SEQ ID NO:873	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTTCTTCAGGG GGCCATGGTCTTC
SEQ ID NO:874	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTTGCTCCTTC CATCCTTGCTCCTG
SEQ ID NO:875	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAAAGCTGTAC CATACCTGTCTG
SEQ ID NO:876	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAAAGTCCCTC TGCTGGTCTGGC
SEQ ID NO:877	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAAGATAATGA CTCACCTGGGGCCA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:878	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGACAACCACCC TTAACCCCTCCTC
SEQ ID NO:879	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGACGTGCCTCT CCCTCCCTC
SEQ ID NO:880	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGCCTGCCGA GATTCCACAGTG
SEQ ID NO:881	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGCGTCATCT GCCCCCAC
SEQ ID NO:882	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGGTTCTGAG CCCCCTTCCG
SEQ ID NO:883	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGAGTCCTGGCG CTGTGTCCTTTC
SEQ ID NO:884	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGATGGCTGGTG TGGTTTGGTTTGTG
SEQ ID NO:885	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCAACCCCCAC AGGCCC
SEQ ID NO:886	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCCCAGCCTCG ACTCGGTTTC
SEQ ID NO:887	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCCCTAATCAC CACCCCACCC
SEQ ID NO:888	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCGAGGGGGGC GTCAG
SEQ ID NO:889	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGCTGTACATGG CCACTCAGATCTCG
SEQ ID NO:890	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGAAAAATGGC TTTGAATCTTTGGC
SEQ ID NO:891	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGAGGATGTGC GGCTCGTA
SEQ ID NO:892	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGATGGAACTG ATGTCTGGACGCTC
SEQ ID NO:893	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGATGGGGTGA GTTTGAGGGAGG
SEQ ID NO:894	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGATGGTCAGC GCACTC
SEQ ID NO:895	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGATGGTCAGC GCACTCTTG
SEQ ID NO:896	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGCCTTCTCCT TTACCCCTCCTTC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:897	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGCTAGCTGTG GGGTGGAGAG
SEQ ID NO:898	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGCTGTGTCCT GGGCTCG
SEQ ID NO:899	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGGGCTCGGGT TGGCTCTAAAG
SEQ ID NO:900	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGGTAGCAAAC TTCTGTACACA
SEQ ID NO:901	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGGTAGCAAAC TTCTGTACACAACT
SEQ ID NO:902	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGGTGCTGATA CTTCTCTCCATCCT
SEQ ID NO:903	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGGTGTGGTTTG GTTTGTGGTCCTC
SEQ ID NO:904	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTATCAGTCTG TCCAGCACTTCCAT
SEQ ID NO:905	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTCATGTAGCA GCTTTCAGGGGC
SEQ ID NO:906	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTCCTCTTCTC CTTCATCGTCTCGG
SEQ ID NO:907	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGATAGGAAG CTGTGGAGTGATG
SEQ ID NO:908	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGCTGCGAGG GGGGC
SEQ ID NO:909	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGTCCCTGTC CTGCCCCC
SEQ ID NO:910	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGTGCTCAGG GGGCCT
SEQ ID NO:911	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTGTGTGTTGATC AGGCGCCCAG
SEQ ID NO:912	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTACTCATTGGG TGGCCGGGC
SEQ ID NO:913	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCACGCTCCTT CCCTATCCCTTCTG
SEQ ID NO:914	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCCAGTGTGA TTGCAGGTTCCAC
SEQ ID NO:915	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCCCACCAGC TTTCCTAATTG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:916	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCCCTCCCCTC GAAATGAAGCTACA
SEQ ID NO:917	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTCCAGGACC ACGGACTGCAC
SEQ ID NO:918	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTGTCAAAGT GGGGGTTCGGAGA
SEQ ID NO:919	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTTCTCATCG CGGGCTTGGTTCTG
SEQ ID NO:920	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTCTTGTCCTGC TTGCTTACCTCGCT
SEQ ID NO:921	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGAATGACCCT GTTAATCCGTTCGT
SEQ ID NO:922	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGAGGTCTCCC CCCGCCATG
SEQ ID NO:923	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGCAGTCGTCA GCCTGAACATAACA
SEQ ID NO:924	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTGTGTGGTGAC TGGCATCTGGTAGG
SEQ ID NO:925	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTATTTGTCCC CTTGCCTCCCTTTC
SEQ ID NO:926	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTCCCTCTGCC CTTTTCAAGCCTCT
SEQ ID NO:927	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTGTGACCTTC GGCTTTTTCAACCC
SEQ ID NO:928	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTTCCTCCTCT TCTCCTGGCCTGAG
SEQ ID NO:929	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTTTTCTCAATGA TGCTTGGCTCTGGA
SEQ ID NO:930	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAAATACATAGA GTTTTAATGCATTGTCTCA
SEQ ID NO:931	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAAGATCACATC ACATGAATGGAATAGT
SEQ ID NO:932	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAAGCCTCACCT GTCTACGTTCCCTC
SEQ ID NO:933	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAATGGATGTTC AGGTAGGAGAGACA
SEQ ID NO:934	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAACCTGGAAGCT GTCTCCACCCA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:935	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAACGCCTCCCCG AGTGAGCTG
SEQ ID NO:936	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAACGTGCTGGTC AAGAGTCCCAAC
SEQ ID NO:937	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAACGTGCTGGTC AAGAGTCCCAAC
SEQ ID NO:938	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGATCCCCTGC CCTCCCCAG
SEQ ID NO:939	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGGGACAGAAG ATGACAGGGGCC
SEQ ID NO:940	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGGTGAAGGTG CTTGGATCTGGC
SEQ ID NO:941	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGTCCTCGTTG TCTTGTTGGCAGGG
SEQ ID NO:942	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGTCCTCGTTG TCTTGTTGGCAGGG
SEQ ID NO:943	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAAGTGCCCTTGG TTCGGACAGAC
SEQ ID NO:944	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAATGCGGGCGAT CTGGGACTG
SEQ ID NO:945	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAATTTTCTGAAC TATTTATGGACAACAGTC
SEQ ID NO:946	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACAAACTCTACG TCTCCTCCGACCAC
SEQ ID NO:947	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACAAGAGGATGG CTAGGCGAGGAG
SEQ ID NO:948	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACAAGCAGCCAC ACCCCATTCTT
SEQ ID NO:949	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACACTCTAGTAT CTGGAAAAATGGCT
SEQ ID NO:950	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACAGAAGATGAC AGGGGCCAGGAG
SEQ ID NO:951	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACAGTAACTTGG GCTTTCTGACGGGA
SEQ ID NO:952	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACATCTGGAGCA TGGGACTGTCTCTG
SEQ ID NO:953	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACCCACTCTGTC TCCGCA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:954	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACCCTAGCCTTA GATAAAACTGAGCA
SEQ ID NO:955	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACGAGGCGGGCA GTGTGTATG
SEQ ID NO:956	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTCTGTCCTGC GTCATCATCTTTGT
SEQ ID NO:957	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTGTGATGAGG TGCCGTTCCCAT
SEQ ID NO:958	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGACTTCCCTTTCC GAATGCCAAACACC
SEQ ID NO:959	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGACCACCACCC TAACCCCAGTCAG
SEQ ID NO:960	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCCAAGGGTGT GAGTGAACGGTG
SEQ ID NO:961	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCCACGATGCC CAGTCAATCTTG
SEQ ID NO:962	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGCTGCCATCTC ACCAAACTGCA
SEQ ID NO:963	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGATGGCAGCG ACGTGGG
SEQ ID NO:964	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGCAGGGCTGT GTCCACC
SEQ ID NO:965	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGCTGGGTGGA GTGGTGTCTAG
SEQ ID NO:966	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGGCCCAGGAG AGTTGCGG
SEQ ID NO:967	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGGGAAAAATA TGACAAAGAAAGCTAT
SEQ ID NO:968	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGGGGAGTCTG GGAATGAACACTAA
SEQ ID NO:969	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGTCTCGATGT AGGGGATGCCG
SEQ ID NO:970	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGGTCTCGATGT AGGGGATGCCGTAG
SEQ ID NO:971	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTACAGAGTGA CCGCCTCAAGTGAC
SEQ ID NO:972	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTGGGTTTATA TTAAAAAGTTGGTCTACT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:973	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTTCCTCAAAA GAGAAATCACGCAT
SEQ ID NO:974	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGAGTTTACCAAAT GTACTCAAGGCATAA
SEQ ID NO:975	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCATGTCTCGG CTCAAGGACCCAAA
SEQ ID NO:976	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCCACTTCCTT GCCCTGCTCAG
SEQ ID NO:977	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCTCCTTGGTG ACCGCTCTGCAT
SEQ ID NO:978	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCTTGTAGGGG ATGTTGAGGCTGCC
SEQ ID NO:979	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATCTTTGTTCCT TCCATTCTTATAGAGC
SEQ ID NO:980	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGGCCAACTCC CCTTCACACCTG
SEQ ID NO:981	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGGGGCCACAC TTACTCTGCAC
SEQ ID NO:982	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATGTTTTTCCGC GGCACCTCCTTC
SEQ ID NO:983	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATTTACCTTTCC TCTGTGTTGGC
SEQ ID NO:984	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGATTTACCTTTCC TCTGTGTTGGCGGA
SEQ ID NO:985	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAACAGCACA GTGAAAGCCAGC
SEQ ID NO:986	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACAAACCTG GCTAAACGTCGGT
SEQ ID NO:987	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAACACAGGGC CAAAGACTAAGTGA
SEQ ID NO:988	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAGAATCAGA ACAATGCCTCCACG
SEQ ID NO:989	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAGACAAATG GTGAGTACGTGCAT
SEQ ID NO:990	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAAGACAAATG GTGAGTACGTGCAT
SEQ ID NO:991	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAATACACAAA GAAAGCCCTCCCCA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:992	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAACACCCAGCC CTCGGT
SEQ ID NO:993	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAACACCCAGCC CTCGGTAAG
SEQ ID NO:994	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAACATCAGAGC TGGATCTAGAAATGG
SEQ ID NO:995	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAAGAAAATACC CCCTCCATCAACTTCT
SEQ ID NO:996	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACACAGATACA GATGTTTTGGAAGCA
SEQ ID NO:997	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACAGCTTTTCC TCCATGAGTACGTA
SEQ ID NO:998	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACAGCTTTTCC TCCATGAGTACGTATTT
SEQ ID NO:999	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCACTCTGACATA TGGCCATTTCTGTTTT
SEQ ID NO:1000	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGAAAGACTTG AAGGCGTATACAGGAAC
SEQ ID NO:1001	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGAGCTAGCTA CTACTGGATTTTT
SEQ ID NO:1002	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGAGCTAGCTA CTACTGGATTTTTGCA
SEQ ID NO:1003	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCATCTCAGG GCCAAAAATTTAAT
SEQ ID NO:1004	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCATCTCAGG GCCAAAAATTTAATCAG
SEQ ID NO:1005	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCCAGAAATA TCCTCCTTACTCATGG
SEQ ID NO:1006	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGCCTACAGAG TCCGCAAGC
SEQ ID NO:1007	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGGTACCGTGC GACATC
SEQ ID NO:1008	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCAGTCGTCAGCC TGAACATAACATCC
SEQ ID NO:1009	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATAATTGAGAG AAAAACTGATATATTAAATGACA
SEQ ID NO:1010	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATAGTAAGCAG TAGGGAGTAACAAAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1011	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGGTGAGGGC TGAGGT
SEQ ID NO:1012	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGGTGAGGGC TGAGGTGAC
SEQ ID NO:1013	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGGTGAGGGC TGAGGTGAC
SEQ ID NO:1014	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGTAACTTCC TGTAATTTTTCAAGGC
SEQ ID NO:1015	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGTATGTTGG CCTCCTTTGCTGC
SEQ ID NO:1016	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGTGGGAGCT AGAAGTGACGT
SEQ ID NO:1017	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCATGTGGGAGCT AGAAGTGACGTCTA
SEQ ID NO:1018	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAAGCCTCACA CCACCCC
SEQ ID NO:1019	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACACACCACC CCTCTGC
SEQ ID NO:1020	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACACATGCCA TCATTCTAGGAAGC
SEQ ID NO:1021	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACCCTGCCAT GCTACCTAGATAC
SEQ ID NO:1022	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACGGGTAATA ATTTTTGTCCTTTC
SEQ ID NO:1023	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACGGGTAATA ATTTTTGTCCTTTCT
SEQ ID NO:1024	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACGGGTAATA ATTTTTGTCCTTTCTGT
SEQ ID NO:1025	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACTGACAACC ACCCTTAACCC
SEQ ID NO:1026	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACTGCCGCTT CCCCAC
SEQ ID NO:1027	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCACTTCTTACC TTCACAGCCACTTG
SEQ ID NO:1028	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGAAATATCC TCCTTACTCATGGTCG
SEQ ID NO:1029	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGACCCAGCC AGTATTATTTCATT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1030	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGACCTAAGA GCAATCAGTGAGGA
SEQ ID NO:1031	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGATCCAGTG AAAAACAAGCTCTCAT
SEQ ID NO:1032	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGTACCTTCC TCTTCTTCTACATCAC
SEQ ID NO:1033	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGTTAACGTC TTCCTTCTCTCTCTGT
SEQ ID NO:1034	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCAGTTAACGTC TTCCTTCTCTCTCTGT
SEQ ID NO:1035	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCATCTTATTCC AGACGCATTTCCACA
SEQ ID NO:1036	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCACCAAGCAG CCCATCC
SEQ ID NO:1037	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCACGCTCTTC TCACTCATATCC
SEQ ID NO:1038	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCACTCTTGCT CCTTCCATCCTTG
SEQ ID NO:1039	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCATGATAGCC GTCTTTAACAAGC
SEQ ID NO:1040	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCATGATAGCC GTCTTTAACAAGC
SEQ ID NO:1041	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCCAGAACTAA CAGGTTAAGTGCTC
SEQ ID NO:1042	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCCGTTCCATC ATAGCATGCAA
SEQ ID NO:1043	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCGAAGTGTAA GCCCAACTACAGAA
SEQ ID NO:1044	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCTAATCACCA CCCCACCCAAT
SEQ ID NO:1045	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCCTGCAGTGAA TTTTGAAGATTGC
SEQ ID NO:1046	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCGCCGCTGAGC CACTG
SEQ ID NO:1047	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCGGTAGTTGCC CTTCTCGAAC
SEQ ID NO:1048	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCGTCTTTAACA AGCTCTTTCTTTCT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1049	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCAAAGAAAA GCTGCGTGATGATG
SEQ ID NO:1050	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCAACGCCCA TGTCTTTGCA
SEQ ID NO:1051	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCAACGCCCA TGTCTTTGCA
SEQ ID NO:1052	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCAGTAAAGC CACCTCACGAACT
SEQ ID NO:1053	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCCACTTTTG CACAGCCAAGAAC
SEQ ID NO:1054	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCCCAAAATG TTAGGATTACAGGTGTG
SEQ ID NO:1055	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTCTTACCTGG AATTTGGATGTGATTGG
SEQ ID NO:1056	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTGCCTCCACT TCAACCACAG
SEQ ID NO:1057	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTGGGGAGCTG GGGACTC
SEQ ID NO:1058	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTTCGCCTGTC CTCATGTATTGG
SEQ ID NO:1059	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTTCTCCTTTA CCCCTCCTTCCTAG
SEQ ID NO:1060	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTTGGTGTGCA TTCTTCTCTCTCTT
SEQ ID NO:1061	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCCTTTCTTCCCT CCCCTCGAAATGAA
SEQ ID NO:1062	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGACAGATCCGG AATATTGTAGAGAAGC
SEQ ID NO:1063	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGACATGTCTTT CCCCACAATCATACT
SEQ ID NO:1064	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGATGGCCCAGC TCCTCAG
SEQ ID NO:1065	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGCATCTCCCTC AGGTAGTTCAGG
SEQ ID NO:1066	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGCTACTAGAAA CATGATAGAGGTGACA
SEQ ID NO:1067	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGAAGAATGTG TCAGCCTCAAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1068	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGGGTCTCTCGG AGGAAGGAC
SEQ ID NO:1069	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTCGTGGAGAA CAAGTTTGGC
SEQ ID NO:1070	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTGGTAGGGCA TTTAAGTATTGGTT
SEQ ID NO:1071	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCGTGGTAGGGCA TTTAAGTATTGGTTGAT
SEQ ID NO:1072	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTAACCAAGTTC TTTCTTTTGCACAGGG
SEQ ID NO:1073	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCAGATGACAG CCGGTTCTCT
SEQ ID NO:1074	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCCCAGGCTGT TTATTTGAAGAGAGAC
SEQ ID NO:1075	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCCTTCCATCC TTGCTCCTGTCC
SEQ ID NO:1076	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCCTTCCCTAT CCCTTCTGCTCTC
SEQ ID NO:1077	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTCTTCCTGTTT CAGTCCCCATTAAA
SEQ ID NO:1078	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGACACCACGA TACTTGACAATGAAAT
SEQ ID NO:1079	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGAGGAAGGTG AAGGTGCTTGGA
SEQ ID NO:1080	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCTGGAATTG GTGTTGATGACC
SEQ ID NO:1081	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGGCTGATCTA TGTCCCTGAAGCAG
SEQ ID NO:1082	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGGGCATCACT GTAAACCTTGCA
SEQ ID NO:1083	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGGTTTGGGGA AGAGTGGGCTA
SEQ ID NO:1084	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTCCTCTTCT CCTTCATCGTCT
SEQ ID NO:1085	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTGGAGTGAT GAGCTGCCAT
SEQ ID NO:1086	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGTGTCCACCC CCTTACTCATTGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1087	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGACATCAGT TTGCCAGTTGTGCT
SEQ ID NO:1088	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGACATCAGT TTGCCAGTTGTGCT
SEQ ID NO:1089	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTGTAAGTGCC CGAAGTGTAAGC
SEQ ID NO:1090	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTTTTCCTCCAT GAGTACGTATTTTGA
SEQ ID NO:1091	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAAAACTGTGT TGTGGAGTGCAAGT
SEQ ID NO:1092	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAAACACATCC CCCAAAGCCAACAA
SEQ ID NO:1093	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAACCAGGAGCT AATAAAAATAACTTCT
SEQ ID NO:1094	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAACTGCTTTGA CTCCAGGTATTCC
SEQ ID NO:1095	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAACTTACTCTC CAGGCTTAACACAG
SEQ ID NO:1096	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGATCATCTG CTGGCCGTGTG
SEQ ID NO:1097	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGCACACAGA TCAGCGACAGGAT
SEQ ID NO:1098	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGCACACAGA TCAGCGACAGGAT
SEQ ID NO:1099	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGCCCTCATG TCTGAACTCAAAGT
SEQ ID NO:1100	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAAGTTTAGGTC AAAGAGGCTGCTTGG
SEQ ID NO:1101	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAATAGGCCTTG GTGTGCATTCTTCT
SEQ ID NO:1102	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAATTTGGATGT GATTGGAAAGTGGGGT
SEQ ID NO:1103	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACATGCTAGGG ACAACACGATTTCC
SEQ ID NO:1104	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACCCTGACAAA TGTGCTGTTCTTCT
SEQ ID NO:1105	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACCGACCGTGA TCAGATTAGGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1106	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTAGGCGTGG GATGTTTTTGCAG
SEQ ID NO:1107	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGACTTCCTCTTC TGCCCTCCCAG
SEQ ID NO:1108	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGAAAAGGGGA CATGCTAGGGACA
SEQ ID NO:1109	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGAACAAGTTT GGCAGCATCCGG
SEQ ID NO:1110	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGACCAGGTAG AGGGAGTACAGAGT
SEQ ID NO:1111	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGACGTTGGAA TGCGGGGAC
SEQ ID NO:1112	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGAGACATTTA AGGTTCCTTCAAGC
SEQ ID NO:1113	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGTACTTCTTT GGGTTGACTTCTCTGG
SEQ ID NO:1114	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGTGGTCATAA GGCTGGTATAATGT
SEQ ID NO:1115	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGAGTTCCTCTTC CTTCCCCTTCTAG
SEQ ID NO:1116	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATGAGTTTTGT GAAAGGCTGGGGAC
SEQ ID NO:1117	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTCAAAGTCA GTCCCCAGCTACTC
SEQ ID NO:1118	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGATTGCAGATTG GGCCTTGGGG
SEQ ID NO:1119	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAAAGGTGGGC TTGTTGGAAGAAC
SEQ ID NO:1120	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAACAGCTCTT ACCTTGTCTTTCTTCC
SEQ ID NO:1121	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAATTCATTTC CAATCAAACCCACAGAC
SEQ ID NO:1122	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCACATTCCATT CTTACCAAACTCTAAATTT
SEQ ID NO:1123	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGACTCTCTC CTCCCCACTG
SEQ ID NO:1124	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCAGACTCTCTC CTCCCCACTG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1125	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATCAATGTCC TTATTACTTGGGAG
SEQ ID NO:1126	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATCAATGTCC TTATTACTTGGGAGACT
SEQ ID NO:1127	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCATTCTGGGAG CTTCATCTGGACC
SEQ ID NO:1128	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCAGATCCAGT GAAAAACAAGCTCT
SEQ ID NO:1129	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCAGATCCAGT GAAAAACAAGCTCT
SEQ ID NO:1130	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCGAAGTCTGA CCCTTTTTGTC
SEQ ID NO:1131	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTCCCAAAAT GTTAGGATTACAGGT
SEQ ID NO:1132	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTCGATCTTG TAGGGGATGTTGAG
SEQ ID NO:1133	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCCTGCCTCCAC TTCAACCA
SEQ ID NO:1134	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCGATCTGGGAC TGCATGCTG
SEQ ID NO:1135	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTCATCACCAC GCTCCATTATCC
SEQ ID NO:1136	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTGCTGGACAT TGACGAGACAGA
SEQ ID NO:1137	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGCTGTGTCCACC CCCTTACTCAT
SEQ ID NO:1138	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGAAGCAGGATC TCAGGTCTCTCAAA
SEQ ID NO:1139	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGAATGCCTGGT TTATTTGGGACTCC
SEQ ID NO:1140	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGACGTGCACAA CCTCGACTA
SEQ ID NO:1141	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGACGTGCACAA CCTCGACTACTA
SEQ ID NO:1142	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGACTAGGCGTG GGATGTTTTTG
SEQ ID NO:1143	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGAGTTGGGGTG AGGGTGTCT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1144	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCAAATGAGTC ACCCGCTATGT
SEQ ID NO:1145	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCAGCATTGTT GGGGGACAC
SEQ ID NO:1146	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCGGGTCTCTC GGAGGAAG
SEQ ID NO:1147	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTCGGGTTGG CTCTAAAGTAGT
SEQ ID NO:1148	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTGAACATA CTAAATGCTCCAGTACT
SEQ ID NO:1149	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTTCTCGGT TCTCTGATTCCTGG
SEQ ID NO:1150	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTTTGTTTT CTTCCCTTTAGATGC
SEQ ID NO:1151	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTTTGTTTT CTTCCCTTTAGATGC
SEQ ID NO:1152	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTTTGTTTT CTTCCCTTTAGATGCTCT
SEQ ID NO:1153	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGCTTTTGTTTT CTTCCCTTTAGATGCTCT
SEQ ID NO:1154	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGAAGCAGGAT CTCAGGTCTCTC
SEQ ID NO:1155	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGACGATGGGG CAAGTGATG
SEQ ID NO:1156	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGATCCTGTCG GTGAGCACT
SEQ ID NO:1157	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGATGTGATGA GAGGTGGATGGG
SEQ ID NO:1158	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGCAAATTTTT AAAGGCACAAGAGGC
SEQ ID NO:1159	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGCTGGGCATC ACTGTAAACCTT
SEQ ID NO:1160	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGCTTTCTCG GTTCTCTGATTCC
SEQ ID NO:1161	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGGGCTTTCTC GGTTCTCTGAT
SEQ ID NO:1162	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGTGGCTATGT AGAGAAGTTGTCCT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1163	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGGTGGTCTTTG GGATCCTCATCAAG
SEQ ID NO:1164	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGTGGGGGGCTC TCACTGTC
SEQ ID NO:1165	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGGTTGTAGTCGG TCATGATGGTCGAG
SEQ ID NO:1166	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAACCATTTAT TTGTTCTCTCTCCAGA
SEQ ID NO:1167	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTACTGCCCTAT TGCCCCTGG
SEQ ID NO:1168	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTACTTAGATGG GGGATGGCTGTTGT
SEQ ID NO:1169	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTAGAGATGGCG GTTGGGAGGTATC
SEQ ID NO:1170	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTATTCGATGAT CCCTGTGGTGG
SEQ ID NO:1171	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCAAAATTAGA ACAGTAGATGCTTAGT
SEQ ID NO:1172	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCAAGAGTCCC AACCATGTCAAAATTACA
SEQ ID NO:1173	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCTCACTCACC CGCGGAC
SEQ ID NO:1174	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTCTCTCGGAGG AAGGACTTGAGGT
SEQ ID NO:1175	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGACCGAGGAC AACGTGATGAAG
SEQ ID NO:1176	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGAGAAAGTTA AAATTCCCGTCGCT
SEQ ID NO:1177	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGAGCACTGAG GGAATGAAAGT
SEQ ID NO:1178	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGCGCATGTAC TGGTCCCG
SEQ ID NO:1179	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGGCTATGTAG AGAAGTTGTCCTGGA
SEQ ID NO:1180	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGGCTTTTTGT TTGTTTGTTTTGTTTTAAGG
SEQ ID NO:1181	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTGTGTCTTTAA TTGAAGCATGATTTAAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1182	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCCTTCAAGC TGCCCTATTGTTAC
SEQ ID NO:1183	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTCTTTGGGGG CAGAGGGGAG
SEQ ID NO:1184	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTGACTGGGCA GAGTGACGATGAG
SEQ ID NO:1185	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTGTCCGGAGC CTAGTCA
SEQ ID NO:1186	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGGTTGTCCGGAGC CTAGTCAAGC
SEQ ID NO:1187	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAAAGCTGGAAA GGGACGAACTGGTG
SEQ ID NO:1188	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAACGTCCTGTC CTGCCCCTGTC
SEQ ID NO:1189	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAACGTCCTGTC CTGCCCCTGTC
SEQ ID NO:1190	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTACTTACCTCAC TTGCCCAGCGTGTC
SEQ ID NO:1191	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTAGAGGGAGTAC AGAGTGACCGCCTC
SEQ ID NO:1192	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAACCACCCAC ATGTCATCAAA
SEQ ID NO:1193	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAAGTACTTAC CCACTGAAAAGCAC
SEQ ID NO:1194	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAGTCCCCAGC TACTCTCAAAATCA
SEQ ID NO:1195	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCATGTGCCCC TCCTTCTGG
SEQ ID NO:1196	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCCAACCATGA CAAGATTTTCCCTT
SEQ ID NO:1197	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCCTTACTTGT TCAGCTCCTTG
SEQ ID NO:1198	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTCAAAAGAC TTGGTGTTGTTGAT
SEQ ID NO:1199	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTCCACACTT CTCCATTCTTCACA
SEQ ID NO:1200	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTGAGCCTGC CGAGATTCCAC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1201	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTGAGCCTGTTTTGTGTCTACTGT
SEQ ID NO:1202	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCCTGGGATTGCAGATTGGGCC
SEQ ID NO:1203	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCAACCCATTCTGCCCAG
SEQ ID NO:1204	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTCCTCCACCGCTTCTTGTCCTG
SEQ ID NO:1205	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCTGCCGCAAATTCCGAGACGAAG
SEQ ID NO:1206	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGACTATCTCCCTGGGTGTAGCT
SEQ ID NO:1207	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGACTATCTCCCTGGGTGTAGCTTTT
SEQ ID NO:1208	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGAGTGGGTTTATATTAAAAAGTTGGT
SEQ ID NO:1209	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGCCACGATGCCCAGTCAATC
SEQ ID NO:1210	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGCCACTTCTTACCTTCACAGC
SEQ ID NO:1211	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGAGCGAGTCCCACAGTGAGGA
SEQ ID NO:1212	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGATCCTCCTGCCTTGGCCTCTATTA
SEQ ID NO:1213	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGATCTGGCTCGTCTGTGTGTGTCA
SEQ ID NO:1214	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGATGGATTTGATGAATTGGTGATAAGA
SEQ ID NO:1215	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGATTGCAGGTTCCACACACAGGC
SEQ ID NO:1216	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCAACGGGTTCCTTCCTTCGAGAG
SEQ ID NO:1217	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCACGAAGGGCCAGGGTATGTG
SEQ ID NO:1218	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCCTCTCCCTCCCTCCAGG
SEQ ID NO:1219	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCTATTTTTCCTCACAGCTCGTTCA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1220	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCTCCAGACCC CTCACCTG
SEQ ID NO:1221	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCTGCTGTACA TGGCCACTCAGATC
SEQ ID NO:1222	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGCTGTGTCCCT GTCCTGCC
SEQ ID NO:1223	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGAGCCTCTTA CACCCAGT
SEQ ID NO:1224	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGAGCCTCTTA CACCCAGT
SEQ ID NO:1225	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGCGCATCTCC CTCAGGTAGTTC
SEQ ID NO:1226	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGCTCCAGTCT CCCTCCTGTTTG
SEQ ID NO:1227	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGGAAGCAG GATCTCAGGTC
SEQ ID NO:1228	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGGGCTCTC ACTGTCTCC
SEQ ID NO:1229	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGGGCTTTC TCGGTTCTCT
SEQ ID NO:1230	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGTGGTCAGC TGCAACATGG
SEQ ID NO:1231	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTACTTACCTC ACTTGCCCAGCGT
SEQ ID NO:1232	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTCTTCCCACC TACAGTAACAAAG
SEQ ID NO:1233	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGAGTGAATG TGTGCCAGGGGTA
SEQ ID NO:1234	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGGATGGAGG GGCACTGAAGTC
SEQ ID NO:1235	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTGTGGCGCTG AGTGTACTTACCTC
SEQ ID NO:1236	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTTGATCAGGC GCCCAGTCAC
SEQ ID NO:1237	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGTTGTGGAGTG CAAGTGAAAGCCTT
SEQ ID NO:1238	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTATCACTCCAC ATTTCAGCAACAGC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1239	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCCCTCAGCCG TTACCTGTGTGTG
SEQ ID NO:1240	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCGCCAGCCAT AAGTCCTCGAC
SEQ ID NO:1241	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCGGACAGACA ACCCCAAGAGCTG
SEQ ID NO:1242	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCTGGGCACTG GGTCAAAGTCTCC
SEQ ID NO:1243	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTCTTTGGGGGC AGAGGGGAGTTG
SEQ ID NO:1244	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGACCGCATCG CCACCTTG
SEQ ID NO:1245	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGGGGTTGTAG TCGGTCATGATG
SEQ ID NO:1246	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTGTCCGGAGCC TAGTCAAGCCTG
SEQ ID NO:1247	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTGTGATGGTT GGGAGGCTGTGTG
SEQ ID NO:1248	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTTCCTCCAAA TACTGACAGCCACA
SEQ ID NO:1249	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAACCCACCTTCT GTCCCACCCCTTC
SEQ ID NO:1250	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAACCTTTTCTTA TGTGCTTTTAGGGC
SEQ ID NO:1251	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAACTCAGCAGCA TCTCAGGGCCAAAA
SEQ ID NO:1252	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAAGTCCTGAGCC TGTTTTGTGTCTAC
SEQ ID NO:1253	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAATGACTCACCT GGGGCCACATTTGA
SEQ ID NO:1254	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAATTTTGCCCAG TTCAGGATCCAGCC
SEQ ID NO:1255	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACACACACGCAA AATACTCCTTCAGC
SEQ ID NO:1256	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCAAATGTACT CAAGGCATAAAAGC
SEQ ID NO:1257	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCACAGTTGCA CAATATCCTTTTGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1258	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCTCAGTTTGC CCCCATGTCCCTTA
SEQ ID NO:1259	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCTCAGTTTGC CCCCATGTCCCTTA
SEQ ID NO:1260	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACCTTCAGCTGC CACTTCTACGACTT
SEQ ID NO:1261	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTGGAGAAAAG GGGACATGCTAGGG
SEQ ID NO:1262	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTACTTACGCGCCA CAGAGAAGTTGTTG
SEQ ID NO:1263	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGAAACCGAGGT ATGAAATTCGCTGG
SEQ ID NO:1264	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGAAGTGACGTC TAGGGGTGGGGG
SEQ ID NO:1265	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGCAAGGTGAAG TAAGACTCAAATGT
SEQ ID NO:1266	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTAGGGGAAGA TCATCTGCTGG
SEQ ID NO:1267	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTAGTAGGGGAAGA TCATCTGCTGGCCG
SEQ ID NO:1268	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATAGAGCGTGCA GATAATGACAAGGA
SEQ ID NO:1269	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATCTGTATCAAA GAATGGTCCTGCAC
SEQ ID NO:1270	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATCTGTATCAAA GAATGGTCCTGCAC
SEQ ID NO:1271	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTATTTGTCCCCTT GCCTCCCTTTCCAA
SEQ ID NO:1272	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAACTCCTGGC CTCTTGTGATCCTC
SEQ ID NO:1273	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAAGTCAGTCC CCAGCTACTCTCAA
SEQ ID NO:1274	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAACTAAACTTC TAAGATGTGGCAAGA
SEQ ID NO:1275	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAAGGAGATAAG TGATGGAGATGTGA
SEQ ID NO:1276	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAATTTGAGGGG GAGTCTGGGAATGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1277	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACAGACATCCT TGCACATCTCTAGC
SEQ ID NO:1278	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACATGAATGGA ATAGTTTAATAGTTTGGA
SEQ ID NO:1279	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACTCATATCCT CCTCTTTCTGCCCA
SEQ ID NO:1280	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCACTCATATCCT CCTCTTTCTGCCCA
SEQ ID NO:1281	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGCAGCATCTC AGGGCCAAAAATTT
SEQ ID NO:1282	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGCCACGGGTA ATAATTTTTGTCCT
SEQ ID NO:1283	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGCTGCCACTT CTACGACTTCTTCA
SEQ ID NO:1284	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGGAAAACTAC AATGGAGAAAGAAGA
SEQ ID NO:1285	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGGTAGGAGAG ACATTTAAGGTTCC
SEQ ID NO:1286	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGTAACTCTAC ACAGAAAGGGCCCA
SEQ ID NO:1287	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGTCCAGACAT GTAGCTCCTGTGC
SEQ ID NO:1288	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGTCCAGACAT GTAGCTCCTGTGC
SEQ ID NO:1289	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCAGTGTTACTTA CCTGTCTTGTCTTT
SEQ ID NO:1290	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATAGATAAAAG CTAAGTTGCCCCAG
SEQ ID NO:1291	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATATTCGTCCA CAAAATGATTCTGAA
SEQ ID NO:1292	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGATTCGTCA TAGTTGTTGCAAGC
SEQ ID NO:1293	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGGGAATTTA AAGGAGCTGGAAAGA
SEQ ID NO:1294	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATGTACTGGTC CCTCATTGCACTGT
SEQ ID NO:1295	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATTATCTGAGG AGCCGGTCACC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1296	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCATTCTTGAGGA GGAAGTAGCGTGGC
SEQ ID NO:1297	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAATCAAACCC ACAGACTTACCTAAT
SEQ ID NO:1298	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAATGGAAAAG AAATGCTGCAGAA
SEQ ID NO:1299	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCACTGAAGCTG AATATTAATGGCCA
SEQ ID NO:1300	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAGTGAAAAAC AAGCTCTCATGTCT
SEQ ID NO:1301	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCAGTGTTTCTT TTAAATACCTGTTAAGT
SEQ ID NO:1302	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCATAAAGACAG AAGGAGGAGAGACA
SEQ ID NO:1303	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCAAGTTTTCT CCCAAATCCCATTT
SEQ ID NO:1304	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCATTTCTCTT TCAGGTGACATTGA
SEQ ID NO:1305	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCCACAATCAT ACTGCTGACATACA
SEQ ID NO:1306	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTCATTTGGAT AGGCTTGTAAGTGC
SEQ ID NO:1307	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTCATTTGGAT AGGCTTGTAAGTGC
SEQ ID NO:1308	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTCTCTCAACT CCAACAGGAAATCA
SEQ ID NO:1309	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGTAATTTTT CAAGGCTTCAGTCT
SEQ ID NO:1310	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGTGGATTTT TAGGCCCTTGTATT
SEQ ID NO:1311	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTGTTTCAGTC CCCATTAAATGAGG
SEQ ID NO:1312	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTACTCATGG TCGGATCACAAAGA
SEQ ID NO:1313	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCTTTTGAAGAC CATAACCCACCACA
SEQ ID NO:1314	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCGTCCACAAAAT GATTCTGAATTAGC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1315	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAACTCTCTTT GACTGCAGAATCCA
SEQ ID NO:1316	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAATGACTGAG ACAATAATTATTAAAAGGTGA
SEQ ID NO:1317	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACACAGAAAG GGCCCAAATTCACC
SEQ ID NO:1318	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTACGACTTCTT CAACCAGGCTGAGT
SEQ ID NO:1319	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTAGGTGAGAGG CAGTGGTCAGG
SEQ ID NO:1320	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCAATGATGCT TGGCTCTGGAATGC
SEQ ID NO:1321	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCCCTGGGTGT AGCTTTTTAAAAAT
SEQ ID NO:1322	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCCGGAGCAAA CCCCTATGTCCAC
SEQ ID NO:1323	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTCTCCTTTT CCTCCTCTTCTCCT
SEQ ID NO:1324	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTCTCTTGAT TCTGACTCTGGCAA
SEQ ID NO:1325	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTGAAATCAA CGTAGAAGTACTCA
SEQ ID NO:1326	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTCTTGCAGTCG TCAGCCTGAACATA
SEQ ID NO:1327	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGAATTAGCTG TATCGTCAAGGCAC
SEQ ID NO:1328	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGGAAAAGAGT AATTCACACAAGCT
SEQ ID NO:1329	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGTATTTATTT CAGTGTTACTTACCTGTC
SEQ ID NO:1330	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGTGAGTGGGA TTTGTTTTGTGGGC
SEQ ID NO:1331	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTGTGGCCTTGT ACTGCAGAGACAA
SEQ ID NO:1332	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTAGAGCATAG TAAGCAGTAGGGAGT
SEQ ID NO:1333	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTATTCCAGAC GCATTTCCACAGCT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1334	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCAGCTTTCT CCCACTGTATTGAA
SEQ ID NO:1335	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCCCATGATG ATCTGTCCCTCACA
SEQ ID NO:1336	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCCCATGATG ATCTGTCCCTCACA
SEQ ID NO:1337	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCTCTTTAGG GTCGGATTCCAGTT
SEQ ID NO:1338	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTCTGGGTGCT GATACTTCTCTCCA
SEQ ID NO:1339	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTGCCAGAGAC ATGTATGATAAAGA
SEQ ID NO:1340	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTGGACCCATG ACTCAACCTCAGTA
SEQ ID NO:1341	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTCATCCCTT CCTCCCTCTTTCTT
SEQ ID NO:1342	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTCATCCCTT CCTCCCTCTTTCTT
SEQ ID NO:1343	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTCCCCACAA TCATACTGCTGACA
SEQ ID NO:1344	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTGCAGGGGT GGCTATGTAGAGAA
SEQ ID NO:1345	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTTGAAAACA ATGGTGACTACATGG
SEQ ID NO:1346	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCTTTTTACCACA GTTGCACAATATCCT
SEQ ID NO:1347	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAAGAGACGGA GCTGAGGAAGG
SEQ ID NO:1348	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAAGCTTAATT CTACCTTGTAGCCT
SEQ ID NO:1349	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAGACCATAAC CCACCACAGCTAGA
SEQ ID NO:1350	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAAGTCAGAAGG GGGTGCCTTTC
SEQ ID NO:1351	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGACAGTTTGACA GTTAAAGGCATTTCC
SEQ ID NO:1352	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGACATATGGCCA TTTCTGTTTTCCTGT

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1353	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGACATCAGTTTG CCAGTTGTGCTTTT
SEQ ID NO:1354	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGACATCAGTTTG CCAGTTGTGCTTTT
SEQ ID NO:1355	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGACCTTCGGCTT TTTCAACCCTTTTTA
SEQ ID NO:1356	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGAGGTGGATG GGTAGTAGTATGGA
SEQ ID NO:1357	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGCACTGAATC TATAAAGCATGTAAC
SEQ ID NO:1358	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGCATTTGAAG TTTTTATTAGTGATGGA
SEQ ID NO:1359	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGCCACTTCTT ACCTTCACAGCCAC
SEQ ID NO:1360	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGTCTATCGAG TGTGTGCATATGTGT
SEQ ID NO:1361	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGAGTGCAGTTGT TTACCATGATAACG
SEQ ID NO:1362	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATACCCCAGCT CAGATCTTCTCCCC
SEQ ID NO:1363	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATAGTTGCTAA GAACCGGTCACTGA
SEQ ID NO:1364	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATGCGAACAGT GAATATTTCCTTTGA
SEQ ID NO:1365	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATGCTGAGGAA GTGGATTTTGCAGG
SEQ ID NO:1366	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATGGATTTGAT GAATTGGTGATAAGATTA
SEQ ID NO:1367	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATGTCTATGAA GTGTTGTGGTTCCT
SEQ ID NO:1368	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGATTCGTCATAG TTGTTGCAAGCCGA
SEQ ID NO:1369	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCAAGGTTTACA CATTTTAATCCCA
SEQ ID NO:1370	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCACCGCGACCT GGCAG
SEQ ID NO:1371	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCAGAAAGACTT GAAGGCGTATACAGG

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1372	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCAGAAGTCCAG GCTGAAAAGGC
SEQ ID NO:1373	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCAGTTTTTCCT CCTACTCACCATCC
SEQ ID NO:1374	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCATAACAACAA AGAATATGAATATGGATCA
SEQ ID NO:1375	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCAACATGACT TACTTGATCCCCATAA
SEQ ID NO:1376	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCAGTTAACGT CTTCCTTCTCTCTC
SEQ ID NO:1377	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCAGTTAACGT CTTCCTTCTCTCTC
SEQ ID NO:1378	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCAGTTAACGT CTTCCTTCTCTCTC
SEQ ID NO:1379	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCATCATTCTA GGAAGCTCACCATT
SEQ ID NO:1380	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCATGCTACCT AGATACCTTTCCCT
SEQ ID NO:1381	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCCCCAAGAAT CCTAGTAGAATGTT
SEQ ID NO:1382	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTCAATAAGC CAACCATGTCTTTCA
SEQ ID NO:1383	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTCTGGTGCC CCCCG
SEQ ID NO:1384	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCCTGGCTCCCT AATTTTATAGTTTTT
SEQ ID NO:1385	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCGAACAGTGAA TATTTCCTTTGATGA
SEQ ID NO:1386	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCGACAGATCCG GAATATTGTAGAGA
SEQ ID NO:1387	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCGGCTCGTACA CAGGGAC
SEQ ID NO:1388	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTAACCAAGTT CTTTCTTTTGCACA
SEQ ID NO:1389	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTAAGAACCGG TCACTGAAAATGAA
SEQ ID NO:1390	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTCCCAGGCTG TTTATTTGAAGAGA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1391	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGGATTGAAA TTCACTTACACCGG
SEQ ID NO:1392	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTCAGAGTT CAACGTCCTGA
SEQ ID NO:1393	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTGTGAGGGTT TTTTGATGTTACCA
SEQ ID NO:1394	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTTTTCTAACT CTCTTTGACTGCAG
SEQ ID NO:1395	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTTTTCTAACT CTCTTTGACTGCAGAAT
SEQ ID NO:1396	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAAAAGAGTAA TTCACACAAGCTCACC
SEQ ID NO:1397	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAACTAGGTCA GCTGAAGATCCTGT
SEQ ID NO:1398	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAAGCCAAGCC CAGTTCTGG
SEQ ID NO:1399	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAAGTCTATGT GATCAAGAAATCGA
SEQ ID NO:1400	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAATCCAGTGT TTCTTTTAAATACCTGT
SEQ ID NO:1401	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGACCCATGACT CAACCTCAGTATTT
SEQ ID NO:1402	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGACGATTTCAC CCAGACCCATGAA
SEQ ID NO:1403	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAGAAAAATGT GATTGCCTGGGTGT
SEQ ID NO:1404	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAGAAGTTAGA CATGTCAACCTTTT
SEQ ID NO:1405	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGAGTCTGGATG GAAGGACAAAAAGA
SEQ ID NO:1406	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGATCATATTGG CCTGTCTGCTCTTC
SEQ ID NO:1407	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGATGTGATTGG AAAGTGGGGTAAAA
SEQ ID NO:1408	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGATTGAAATTC ACTTACACCGGGCC
SEQ ID NO:1409	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGATTTGATGAA TTGGTGATAAGATTAACA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1410	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAAACAATAC CAAATTTACTTCATGT
SEQ ID NO:1411	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAATTCATTT CCAATCAAACCCACA
SEQ ID NO:1412	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCACATCAAGG GAGGGTTC
SEQ ID NO:1413	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCACATTCCAT TCTTACCAAACTCTAAA
SEQ ID NO:1414	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCACATTCCAT TCTTACCAAACTCTAAA
SEQ ID NO:1415	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAGTCAAACC TTCTCTCTTATGTATA
SEQ ID NO:1416	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCAGTCAAACC TTCTCTCTTATGTATATAT
SEQ ID NO:1417	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCATGGGGAAA TATAAACTTGTTTGA
SEQ ID NO:1418	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCCATGGAACC AGACAGAAAAGC
SEQ ID NO:1419	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCCATTTCTGT TTTCCTGTAGCAAA
SEQ ID NO:1420	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGCTTGATCCTG AGTCATTTCTTCCT
SEQ ID NO:1421	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGAAAAAGTGG TGGTATACGATATGGGT
SEQ ID NO:1422	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGAAGGGACAG AAGATGACAGGG
SEQ ID NO:1423	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGAATTTAAAG GAGCTGGAAAGAGTGC
SEQ ID NO:1424	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGATGTTTTTG CAGATGATGGGCTC
SEQ ID NO:1425	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGCCCCAGAAC TAACAGGTTAAGTG
SEQ ID NO:1426	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGTAAAGATGA TCCGACAAGTGAGA
SEQ ID NO:1427	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGGTAGCAAACT TCTGTACACAACTAAC
SEQ ID NO:1428	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTATTTTTGGT TCTAGATCTTTGTTCC

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1429	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTGGCTTTTTG TTTGTTTGTTTTGT
SEQ ID NO:1430	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTGGCTTTTTG TTTGTTTGTTTTGTTTT
SEQ ID NO:1431	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTACATACTT GGACTTGGTGATAGA
SEQ ID NO:1432	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTCAGAGTTC TATAGATTCTAGTGCA
SEQ ID NO:1433	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTCTAGATCT TTGTTCCTTCCATTC
SEQ ID NO:1434	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTGATATTAT TCTTCTTGTGCCTGGG
SEQ ID NO:1435	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTGTGTTTGG TTTTGTGGGAGTCT
SEQ ID NO:1436	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGGTTTGTTTTGG TTGTGTTTGGTTTT
SEQ ID NO:1437	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAAGTCAAAGG GGTATTCGATGATCC
SEQ ID NO:1438	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAATCAGACGA CACAGGAAGCAGAT
SEQ ID NO:1439	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTACCAACCTCA CCAACATTACAGAG
SEQ ID NO:1440	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTACCAACCTCA CCAACATTACAGAG
SEQ ID NO:1441	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAGAGGTTAAT ATCCGCAAATGACT
SEQ ID NO:1442	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTAGGAGTGGTC ATAAGGCTGGTATAA
SEQ ID NO:1443	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTATTATATAGG GCAGAGTCATGTTAGTC
SEQ ID NO:1444	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTATTCACAGAG ACTTGGCAGCCA
SEQ ID NO:1445	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTATTTAACCAT GCAGATCCTCAGTT
SEQ ID NO:1446	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCAAAAATTGT TTCTGGGGCCATCC
SEQ ID NO:1447	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCAAGCCCTCC AACATCCTAGTCAA

Target Specific Primer	Volume Ratio	Sequence
SEQ ID NO:1448	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCACAGCACCC TAGAACCAAATCCA
SEQ ID NO:1449	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCACCCACATC AAGATTCAGAACAC
SEQ ID NO:1450	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCAGTCTGCCC TTCTGTCAAAGTGG
SEQ ID NO:1451	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCCAGTCACTG TGCTGCTTCA
SEQ ID NO:1452	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCCTGCGTCAT CATCTTTGTCATCG
SEQ ID NO:1453	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCCTTATTACT TGGGAGACTTGTCT
SEQ ID NO:1454	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCTATGAAGTG TTGTGGTTCCTTAA
SEQ ID NO:1455	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCTCACTGCCT CATCTCTCACCATC
SEQ ID NO:1456	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTCTCATCCCAA ATATTCTCCAGGCGT
SEQ ID NO:1457	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGACCTTCGGC TTTTTCAACCCTTT
SEQ ID NO:1458	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGAGTGGGATT TGTTTTGTGGGCTAC
SEQ ID NO:1459	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGATGAGAGGT GGATGGGTAGTAGT
SEQ ID NO:1460	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCATTATTGT GATGATTCTGACCT
SEQ ID NO:1461	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGCATTATTGT GATGATTCTGACCTACA
SEQ ID NO:1462	1	GACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTGGCTACATGT TCCTGATCTCCTTA
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Target Specific Primer	Volume Ratio	Sequence
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