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(54) Titre : SONDES DE CAPTURE IMMOBILISABLES PAR L'INTERMEDIAIRE D'UNE QUEUE NUCLEOTIDIQUE L
(54) Title: CAPTURE PROBES IMMOBILIZABLE VIA L-NUCLEOTIDE TAIL

(57) Abrégé/Abstract:
The invention provides chimeric capture probes immobilizable via an L-nucleic acid tail that can bind to a complementary L-nucleic acid in an immobilized probe. The capture probes are useful for capturing a target nucleic acid from a sample. The L-nucleic acid in the tail of the capture probe bind to the complementary L-nucleic acid in the immobilized probe with similar affinity as would otherwise equivalent D-nucleic acids. However, the L-nucleic acid of the capture probe tail and immobilized probes do not form stable duplexes with D-nucleic acids present in the in the sample containing the target nucleic acid. Binding of nucleic acids in the sample directly to immobilized probe or to the tail of the capture probe is reduced or eliminated increasing the sensitivity and/or specificity of the assay.

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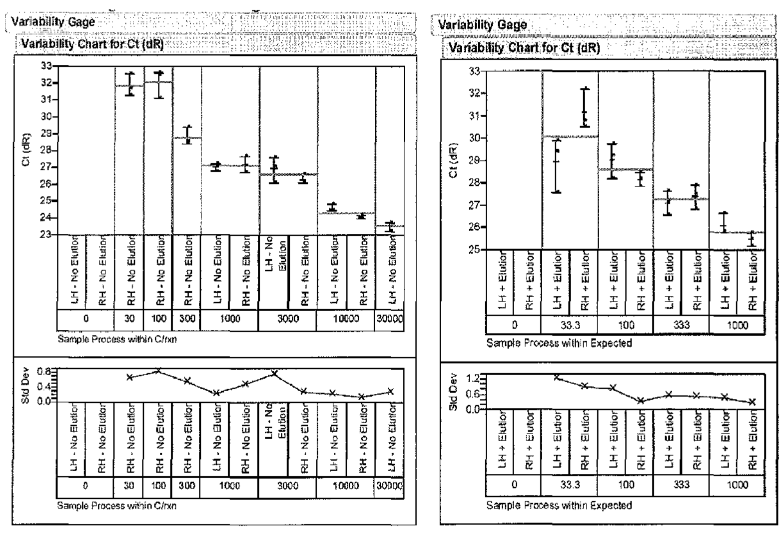
[Continued on next page]

(54) **Title**: CAPTURE PROBES IMMOBILIZABLE VIA L-NUCLEOTIDE TAIL

Fig. 2



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CAPTURE PROBES IMMOBILIZABLE VIA L-NUCLEOTIDE TAIL

CROSS-REFERENCE TO RELATED APPLICATION

[0001] The present application is a nonprovisional and claims the benefit of 61/383,728 filed September 16, 2010.

BACKGROUND

[0002] Detection of nucleic acids in a sample is useful in diagnostic, therapeutic, forensic, agricultural, food science applications and other areas. One technique for purifying a target polynucleotide, which is often used in diagnostic procedures, involves capturing a target polynucleotide onto a solid support. The solid support retains the target polynucleotide during one or more washing steps of the target polynucleotide purification procedure. The captured target sequence can be analyzed by various methods. One such method uses nucleic acid probes that hybridize to a target sequence. Probes can be designed to detect different target sequences such as those characteristic of microorganisms, viruses, human genes, plant or animal genes, and/or pathogenic conditions. Additional analysis techniques that benefit from captured target nucleic acids include, amplification assays, microarrays, sequencing assays, mass spectrometry of nucleic acids, and other techniques known in the art.

[0003] A target nucleic acid can be captured using a mediator or capture polynucleotide that hybridizes to bind both to a target nucleic acid and to a nucleic acid fixed to a solid support. The mediator polynucleotide joins the target nucleic acid to the solid support to produce a complex comprising a bound target nucleic acid. A labeled probe can be hybridized to the bound target and unbound labeled probe can be washed away from the solid support (see Stabinsky, U.S. Pat. No. 4,751,177).

[0004] Because hybridization proceeds more rapidly between nucleic acids that are both in solution compared to between one nucleic acid in solution and one immobilized nucleic acid, it is preferable in capturing a target nucleic acid using a capture probe that the capture probe hybridizes to the target nucleic before the capture probe is immobilized by binding to a support-bound polynucleotide. Such can be accomplished by performing the capture under first hybridization conditions in which the capture probe hybridizes to the target nucleic acid to for a target capture probe:target nucleic acid complex, and second hybridization conditions of reduced stringency in which the capture probe of the formed complex binds to an immobilized probe to form a further complex comprising immobilized probe:target capture probe:target nucleic acid (see Weisburg et al, US Pat. No. 6,110,678). This type of assay is facilitated by designing the capture probe and immobilized probe to contain complementary homopolymer sequences of adenine and thymine. The melting temperature of hybrids formed between adenine and thymine is usually less than that formed between complementary sequences that include guanine and cytosine residues.

SUMMARY OF THE CLAIMED INVENTION

[0005] The invention provides methods of capturing a target nucleic acid. The methods involve contacting a target nucleic acid with a capture probe and an immobilized probe, the capture probe comprising a first segment that binds to the target nucleic acid and a second segment that binds to the immobilized probe, wherein the second segment of the capture probe and the immobilized probe comprise L-nucleic acids that can hybridize to one another, wherein the target nucleic acid binds to the first segment of the capture probe, and the second segment of the capture probe binds to the target, thereby capturing the target nucleic acid. Optionally, the first segment includes a D nucleic acid of at least 10 D-nucleobase units complementary to the target nucleic acid. Optionally, the D-nucleic acid comprises any of adenine-D-deoxyribose, guanine D-deoxyribose, thymine D-deoxyribose and cytosine D-deoxyribose. Optionally, the first segment includes a D-nucleic acid of 10-30 D-nucleobase units complementary to the target nucleic acid. Optionally, the first segment binds non-specifically to the target nucleic acid. Optionally, the first segment includes a random sequence of D-nucleobase units that binds nonspecifically to the target nucleic acid. Optionally, the second segment includes an L-nucleic acid of at least six L-nucleobase units complementary to an L-nucleic acid of at least six L-nucleobase units in the immobilized probe. Optionally, the second segment includes an L-nucleic acid of 10-30 L-nucleobase units complementary to an L-nucleic acid of 10-30 contiguous L-nucleobase units in the immobilized probe. Optionally, the L-nucleic acid of the second segment is a homopolymer and the L-nucleic acid of the immobilized probe constitutes a complementary homopolymer. Optionally, the homopolymer of the second segment is a homopolymer of adenine nucleobase units and the homopolymer of the immobilized probed is a homopolymer of thymine nucleobase units or vice versa. Optionally, the target nucleic acid is contacted with the capture probe and immobilized probe simultaneously. Optionally, the target nucleic acid is contacted with the capture probe before the immobilized probe. Optionally, the binding of the target nucleic acid to the capture probe occurs under first hybridization conditions and the binding of the capture probe to the immobilized probe occurs under second hybridization conditions. Optionally, the first conditions are more stringent than the second conditions. Optionally, the binding of the target nucleic acid to the capture probe and the binding of the capture probe to the immobilized probe occur under the same hybridization conditions. Optionally, the L-nucleic acids comprise any of adenine L-deoxyribose, guanine L-deoxyribose, cytosine L-deoxyribose or thymine L-deoxyribose joined by phosphodiester linkages. Optionally, the immobilized probe is immobilized to a magnetic bead. Optionally, the target nucleic acid is provide as a component of a sample and the method further comprising separating the captured target nucleic acid from other components of the sample. Optionally, the captured target nucleic acid is dissociated from the immobilized probe. Optionally, the target nucleic acid is amplified. The amplifying can be performed after dissociating the target nucleic acid from the capture probe. Optionally, the target nucleic acid is contacted with a detection probe and detecting the detection probe. Optionally, the target nucleic is contacted with the detection probe after dissociating the target nucleic acid from the capture probe. Optionally, the target nucleic acid is an HIV nucleic acid. Optionally, the L-nucleic acids are a homopolymer of adenine nucleobase units and a homopolymer of thymine nucleobase units and the sample includes mRNA or a nucleic acid derived therefrom.

[0006] The invention further provides a capture probe comprising a first segment of at least 10 D-nucleobase units and a second segment comprising a homopolymer of at least 10 L-nucleobase units. Optionally, the second segment is a homopolymer of adenine or thymine nucleobase units, preferably a homopolymer of thymine nucleobase units.

The invention further provides an immobilized probe comprising a homopolymer of at least 10 L-nucleobase units linked to a support. Optionally the support is a magnetic particle. Optionally, the homopolymer is a homopolymer of thymine nucleobase units.

[0007] The invention further provides a kit comprising a capture probe comprising a first segment including a D-nucleic acid that binds to a target nucleic acid and a second segment including a homopolymeric L-nucleic acid and an immobilized probe comprising an L-nucleic acid comprising a complementary homopolymeric L-nucleic acid, wherein the capture probe and immobilized probe can hybridize to one another via hybridization of the complementary homopolymeric L-nucleic acids. Optionally, the homopolymeric L-nucleic acid is a homopolymer of adenine nucleobase units and the complementary homopolymeric L-nucleic acid is a homopolymer of thymine nucleobase units.

[0007A] Various embodiments of the claimed invention relate to a method of capturing a target nucleic acid, comprising: contacting a target nucleic acid with a capture probe and an immobilized probe, the capture probe comprising a first segment that binds to the target nucleic acid and a second segment that binds to the immobilized probe, wherein the second segment of the capture probe and the immobilized probe comprise poly-A and poly-T L-nucleic acids that can hybridize to one another, wherein the first segment of the capture probe binds to the target nucleic acid, and the second segment of the capture probe binds to the immobilized probe with a lower melting temperature than the first segment of the capture probe binds to the target nucleic acid, thereby capturing the target nucleic acid.

[0007B] Various embodiments of the claimed invention relate to a hybridization complex comprising: (i) a capture probe comprising a first segment of at least 10 D-nucleobase units that can hybridize to a target nucleic acid and a second segment comprising a homopolymer of at least 10 L-nucleobase units (ii) immobilized probe comprising a homopolymer of at least 10 L-nucleobase units linked to a support wherein the capture probe and immobilized probe comprise complementary homopolyeric L-nucleic acids that can hybridize to one another, with a lower melting temperature than the first segment of the capture probe binds to the target nucleic acid, wherein the homopolymeric L-nucleic acids are polyA and polyT.

[0007C] Various embodiments of the claimed invention relate to a kit comprising: a capture probe comprising a first segment including a D-nucleic acid that hybridizes to a target nucleic acid and a second segment including a homopolymeric L-nucleic acid and an immobilized probe comprising an L-nucleic acid comprising a complementary homopolymeric L-nucleic acid,, wherein the homopolymeric L-nucleic acids are poly-A and poly-T and can hybridize to one another via hybridization of the complementary homopolymeric L-nucleic acids with a lower melting temperature than the first segment of the capture probe hybridizes to the target nucleic acid.

BRIEF DESCRIPTION OF THE FIGURES

[0008] Fig. 1 compares D- and L-nucleic acids.

[0009] Fig. 2 compares quantitative PCR emergence times for targets captured with different capture probes.

DEFINITIONS

[0010] [0001] A nucleic acid refers to a multimeric compound comprising nucleotides or analogs that have nitrogenous heterocyclic bases or base analogs linked together to form a polymer, including conventional RNA, DNA, mixed RNA-DNA, and analogs thereof.

[0011] The nitrogenous heterocyclic bases can be referred to as nucleobases. Nucleobases can be conventional DNA or RNA bases (A, G, C, T, U), base analogs, e.g., inosine, 5-nitroindazole and others (The Biochemistry of the Nucleic Acids 5-36, Adams et al., ed., 11.sup.th ed., 1992; van Aerschott et al., 1995, Nucl. Acids Res. 23(21): 4363-70), imidazole-4-carboxamide (Nair et al., 2001, Nucleosides Nucleotides Nucl. Acids, 20(4-7):735-8), pyrimidine or purine derivatives, e.g., modified pyrimidine base 6H,8H-3,4-dihydropyrimido[4,5-c][1,2]oxazin-7-one (sometimes designated "P" base that binds A or G) and modified purine base N6-methoxy-2,6-diaminopurine (sometimes designated "K" base that binds C or T), hypoxanthine (Hill et al., 1998, Proc. Natl. Acad. Sci. USA 95(8):4258-63, Lin and Brown, 1992, Nucl. Acids Res. 20(19):5149-52), 2-amino-7-deaza-adenine (which pairs with C and T; Okamoto et al., 2002, Bioorg. Med. Chem. Lett. 12(1):97-9), N-4-methyl deoxygaunosine, 4-ethyl-2'-deoxycytidine (Nguyen et al., 1998, Nucl. Acids Res. 26(18):4249-58), 4,6-difluorobenzimidazole and 2,4-difluorobenzene nucleoside analogues (Kiopffer & Engels, 2005, Nucleosides Nucleotides Nucl. Acids, 24(5-7) 651-4), pyrene-functionalized LNA nucleoside analogues (Babu & Wengel, 2001, Chem. Commun. (Camb.) 20: 2114-5; Hrdlicka et al., 2005, J. Am. Chem. Soc. 127(38): 13293-9), deaza- or aza-modified purines and pyrimidines, pyrimidines with substituents at the 5 or 6 position and purines with substituents at the 2, 6 or 8 positions, 2-aminoadenine (nA), 2-thiouracil (sU), 2-amino-6-methylaminopurine, O-6-methylguanine, 4-thio-pyrimidines, 4-amino-pyrimidines, 4-dimethylhydrazine-pyrimidines, and O-4-alkyl-pyrimidines (U.S. Pat. No.

5,378,825; PCT No. WO 93/13121; Gamper et al., 2004, Biochem. 43(31): 10224-36), and hydrophobic nucleobases that form duplex DNA without hydrogen bonding (Berger et al., 2000, Nucl. Acids Res. 28(15): 2911-4). Many derivatized and modified nucleobases or analogues are commercially available (e.g., Glen Research, Sterling, Va.).

[0012] A nucleobase unit attached to a sugar, can be referred to as a nucleobase unit, or monomer. Sugar moieties of a nucleic acid can be ribose, deoxyribose, or similar compounds, e.g., with 2' methoxy or 2' halide substitutions. Nucleotides and nucleosides are examples of nucleobase units.

[0013] The nucleobase units can be joined by a variety of linkages or conformations, including phosphodiester, phosphorothioate or methylphosphonate linkages, peptide-nucleic acid linkages (PNA; Nielsen et al., 1994, Bioconj. Chem. 5(1): 3-7; PCT No. WO 95/32305), and a locked nucleic acid (LNA) conformation in which nucleotide monomers with a bicyclic furanose unit are locked in an RNA mimicking sugar conformation (Vester et al., 2004, Biochemistry 43(42):13233-41; Hakansson & Wengel, 2001, Bioorg. Med. Chem. Lett. 11 (7):935-8), or combinations of such linkages in a nucleic acid strand. Nucleic acids may include one or more "abasic" residues, i.e., the backbone includes no nitrogenous base for one or more positions (U.S. Pat. No. 5,585,481).

[0014] A nucleic acid may include only conventional RNA or DNA sugars, bases and linkages, or may include both conventional components and substitutions (e.g., conventional RNA bases with 2'-O-methyl linkages, or a mixture of conventional bases and analogs). Inclusion of PNA, 2'-methoxy or 2'-fluoro substituted RNA, or structures that affect the overall charge, charge density, or steric associations of a hybridization complex, including oligomers that contain charged linkages (e.g., phosphorothioates) or neutral groups (e.g., methylphosphonates) may affect the stability of duplexes formed by nucleic acids.

[0015] An oligomer may contain a "random polymer" sequence that refers to a population of oligomers that are substantially the same in overall length and other characteristics, but in which at least a portion of the oligomer is synthesized by random incorporation of different bases for a specified length, e.g., a random assortment of all four standard bases (A, T, G, and C) in a DNA oligomer, or a random assortment of a few bases (U and G) in a defined portion of a larger oligomer. The resulting oligomer is actually a population of oligomers whose finite number of members is determined by the length and number of bases making up the random portion (e.g., 2.sup.6 oligomers in a population of oligomers that contains a 6-nt random sequence synthesized by using 2 different bases).

[0016] Complementarity of nucleic acids means that a nucleotide sequence in one strand of nucleic acid, due to orientation of its nucleobase groups, hydrogen bonds to another sequence on an opposing nucleic acid strand. The complementary bases typically are, in DNA, A with T and C with G, and, in RNA, C with G, and U with A. Complementarity can be perfect (i.e., exact) or substantial/sufficient. Perfect complementarity between two nucleic acids means that the two nucleic acids can form a duplex in which every base in the duplex is bonded to a complementary base by Watson-Crick pairing. "Substantial" or "sufficient" complementary means that a sequence in one strand is not completely and/or perfectly complementary to a sequence in an opposing strand, but that sufficient bonding occurs between bases on the two strands to form a stable hybrid complex in set of hybridization conditions (e.g., salt concentration and temperature). Such conditions can be predicted by using the sequences and standard mathematical calculations to predict the Tm of hybridized strands, or by empirical determination of Tm by using routine methods. Tm refers to the temperature at which a population of hybridization complexes formed between two nucleic acid strands are 50% denatured. At a temperature below the Tm, formation of a hybridization complex is favored, whereas at a temperature above the Tm, melting or separation of the strands in the hybridization complex is favored. Tm may be estimated for a nucleic acid having a known G+C content in an aqueous 1 M NaCl solution by using, e.g., Tm=81.5+0.41(% G+C), although other known Tm computations take into account nucleic acid structural characteristics.

[0017] "Hybridization condition" refers to the cumulative environment in which one nucleic acid strand bonds to a second nucleic acid strand by complementary strand interactions and hydrogen bonding to produce a hybridization complex. Such conditions include the chemical components and their concentrations (e.g., salts, chelating agents, formamide) of an aqueous or organic solution containing the nucleic acids, and the temperature of the mixture. Other factors, such as the length of incubation time or reaction chamber dimensions may contribute to the environment (e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, 2.sup.nd ed., pp. 1.90-1.91, 9.47-9.51, 11.47-11.57 (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989)).

[0018] A "label" refers to a molecular moiety that is detectable or produces a detectable response or signal directly or indirectly, e.g., by catalyzing a reaction that produces a detectable signal. Labels include luminescent moieties (such as fluorescent, bioluminescent, or chemiluminescent compounds), radioisotopes, members of specific binding pairs (e.g., biotin and avidin), enzyme or enzyme substrate, reactive groups, or chromophores, such as a dye or particle that results in detectable color.

[0019] A "detection probe" is a nucleic acid or other molecule that binds specifically to a target sequence and which binding results, directly or indirectly, in a detectable signal to indicate the presence of the target sequence. A detection probe need not be labeled to produce a detectable signal, e.g., an electrical impulse resulting from binding the probe to its target sequence may be the detectable signal. A "labeled probe" is a probe that contains or is linked, directly or indirectly, to a label (e.g., Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd ed., Chapt. 10; U.S. Pat. No. 6,361,945, Becker et al.; U.S. Pat. No. 5,658,737, Nelson et al.; U.S. Pat. No. 5,656,207, Woodhead et al.; U.S. Pat. No. 5,547,842, Hogan et al.; U.S. Pat. No. 5,283,174, Arnold et al.; U.S. Pat. No. 4,581,333, Kourilsky et al.; U.S. Pat. No. 5,731,148, Becker et al.). For example, detection probes may include a non-nucleotide linker and a chemiluminescent label attached to the linker (U.S. Pat. Nos. 5,185,439, 5,585,481 and 5,639,604, Arnold et al.). Examples of detection probes include oligonucleotides of about 5 to 50 nucleotides in length having an attached label that is detected in a homogeneous reaction, e.g., one that uses differential hydrolysis of a label on a bound or unbound probe.

[0020] Detection probes can have a nucleotide sequence that is of the same or opposite sense as the a target sequence depending on the format of the assay. Detection probes can hybridize to the same or different segment of a target sequence as a capture probe. Some detection probes have an attached chemiluminescent marker, e.g., an acridinium ester (AE) compound (U.S. Pat. Nos. 5,185,439, 5,639,604, 5,585,481, and 5,656,744). In some detection probes, an acridinium ester label is attached to a central region of the probe near a region of A and T base pairs by using a non-nucleotide linker (U.S. Pat. Nos. 5,585,481 and 5,656,744, Arnold, et al.) which restricts the amines of the nucleotide bases on both sides of the AE and provides a site for intercalation. Alternatively, an AE label may be attached to the 3' or 5' terminus of the detection probe which is used in conjunction with a second oligomer that hybridizes adjacent to the detection probe on the target nucleic acid to restrict the effects of nearby amine contributed by the target nucleic acid. In some detection probes, an AE label at or near the site of a mismatch with a related non-target polynucleotide sequence, to permit discrimination between the related sequence and the target sequence that may differ by only one nucleotide because the area of the duplex around the mismatch site is sufficiently destabilized to render the AE on the probe hybridized to the related non-target sequence susceptible to hydrolysis degradation. HIV 1 and HCV may be detected using a modified form of the commercial PROCLEIX® HIV-1/HCV Assay from Gen-Probe. The modification involves replacing the D-polyA and D-polyT sequences in capture and immobilized probes with L-poly A and L-poly-T, respectively.

[0021] Specific binding of a capture probe to a target nucleic or target nucleic acids means binding between a single defined sequence in the first segment of a capture probe and an exactly or substantially complementary segment on target nucleic acid(s) to form a stable duplex. Such binding is detectably stronger (higher signal or melting temperature) than binding to other nucleic acids in the sample lacking a segment exactly or substantially complementary to the single defined capture probe sequence. Non-specific binding of a capture probe to target nucleic acids means that the capture probe can bind to a population of target sequences that do not share a segment having exact or substantial complementarity to a single defined capture probe sequence. Such can be achieved by for example using a randomized sequence in the first segment of the capture probe.

[0022] Lack of binding as between an L-nucleic acid and D-nucleic acid can be manifested by binding indistinguishable from nonspecific binding occurring between a randomly selected pair of nucleic acids lacking substantial complementarity but of the same length as the L and D nucleic acid in question. Lack of binding between an L-nucleic acid and D-nucleic acid can also be manifested by lack of a detectable TM and/or capture of less than 1% of a target nucleic acid or a signal of captured target indistinguishable within experimental error from that captured by a control lacking a capture probe when the L-nucleic acid and D-nucleic acid are used to pair with one another in the capture probe and immobilized probe (or vice versa).

[0023] "Homogeneous detectable label" refers to a label whose presence can be detected in a homogeneous manner depending on whether the label is bound or unbound to a target. A homogeneous detectable label can be detected in a "homogeneous reaction" without physically separating unbound forms of the label from the mixture before the detection step. A homogeneous reaction may occur in solution or on a support such as a microarray, biochip, or gene chip. Preferred homogeneous detectable labels and conditions for their detection have been described previously in detail (U.S. Pat. No. 5,283,174, Arnold et al.; U.S. Pat. No. 5,656,207, Woodhead et al.; U.S. Pat. No. 5,658,737, Nelson et al.).

[0024] A "chimeric capture probe" serves to join a target nucleic acid and an immobilized probe by hybridization of complementary sequences. A chimeric target capture probe is sometimes referred to as a capture probe. A chimeric capture probe includes a first segment including a target-complementary region of sequence and a second segment for attaching the capture probe, or a hybridization complex that includes the capture probe, to an immobilized probe. The first segment can be configured to substantially complementary to a specific target nucleic acid sequence so that a first segment and a target nucleic acid can hybridize to form a stable duplex (i.e., having a detectable melting point) under hybridizing conditions, such as described in the Examples. Alternatively, the first segment can be configured to nonspecifically bind to nucleic acid sequences in a sample under hybridizing conditions (see WO 2008/016988). The second segment includes a region of sequence that is complementary to a sequence of an immobilized probe. Preferably, a chimeric capture probe includes a nucleic acid homopolymer (e.g., poly-A or poly-T) that is covalently attached to the target-complementary region of the capture probe and that hybridizes under appropriate conditions to a complementary homopolymer of the immobilized probe (e.g., poly-T or poly-A, respectively) as previously described (U.S. Pat. No. 6,110,678 to Weisburg et al.). Capture probes may further comprise a third segment that acts as a closing sequence to inactivate unbound target capture probes in a capture reaction. This third segment can flank the first segment opposite the second segment (e.g., capture sequence:target hybridizing sequence:closing sequence) or it can flank the second segment opposite the first segment (e.g., closing sequence:capture sequence:target hybridizing sequence). See WO 2006/007567 and US 2009-0286249.

[0025] "Separating" or "isolating" or "purifying" refers to removing one or more components from a complex mixture, such as a sample. Preferably, a separating, isolating or purifying step removes at least 70%, preferably at least 90%, and more preferably about 95% of the target nucleic acids from other sample components. A separating, isolating or purifying step may optionally include additional washing steps to remove non-target sample components. It is understood that at least X% refers to a range from X% to 100% inclusive of all whole and partial numbers (e.g., 70%, 82.5%, etc.)

[0026] "Release" of a capture hybrid refers to separating one or more components of a capture hybrid from each other, such as separating a target nucleic acid from a capture probe, and/or a capture probe from an immobilized probe. Release of the target nucleic acid strand separates the target from other components of a capture hybrid and makes the target available for binding to a detection probe. Other components of the capture hybrid may remain bound, e.g., the capture probe strand to the immobilized probe on a capture support, without affecting target detection.

[0027] Sensitivity is the proportion of true positives correctly identified as such (e.g. the percentage of infected patients correctly identified as having the infection). Specificity measures the proportion of true negatives which are correctly identified (e.g. the percentage of uninfected patients who are correctly identified as not having the infection.)

DETAILED DESCRIPTION

I. General

[0028] The invention provides chimeric target capture probes comprising first segments and second segments, wherein the capture probes are immobilizable to a support bearing an immobilized probe that is complementary to the second segment, and wherein the complementary portions of the second segment and the immobilized probe comprise left-handed nucleobase units forming an L-nucleic acid. L-nucleic acids are not capable of hybridizing to natural (i.e., D-) nucleic acids. Employing the chimeric capture probe with a D-nucleic acid first segment and an L-nucleic acid second segment for immobilization to an L-nucleic acid containing immobilized probe enables more specific capture of an intended target nucleic acid. For example, mRNAs, non-target virus sequences, or other such unintended non-target nucleic acid sequences can be prevented from binding to L-(polyT) particles via their comprised polyA sequences (either terminal or internal). This is particularly useful for specific capture of nucleic acids containing poly-A sections using a poly-A/poly-T capture configuration (e.g., US Pat. No. 6,110,678) (e.g., fragmented genomic loci, mRNAs or viral nucleic acids (i.e. to prevent unintended capture of other loci that contain strings of polyA)).

[0029] The capture probes of the current invention are useful for capturing a target nucleic acid from a sample. The L-nucleic acid in the tail of the capture probe bind to the complementary L-nucleic acid in the immobilized probe with similar affinity as would otherwise equivalent D-nucleic acids. However, the L-nucleic acid of the capture probe tail and immobilized probes lack affinity for D-nucleic acids present in the in the sample containing the target nucleic acid.

Accordingly, binding of nucleic acids in the sample directly to immobilized probe rather than via the capture probe as an intermediate can be eliminated. Binding of the capture probe's second segment to nucleic acids in the sample can therefore also be eliminated. Avoiding direct binding of nucleic acids to the immobilized probe and/or to the capture probe's second segment offers advantages in increasing the sensitivity and/or specificity of analytical techniques that use the captured target nucleic acids. The purity of target nucleic acid is particularly advantageous for subsequent sequencing analyses, particularly by methods involving massively parallel sequencing of multiple different target nucleic acids including variants. Such methods of sequencing include single-molecule real time methods discussed further below. The methods are particularly useful when homopolymers of adenine and thymine are used in the capture probe tail and immobilized probe and the sample contains poly-A and/or poly-T containing nucleic acids (for example a sample containing mRNA or cDNA, viral RNA, human nucleic acids, mitochondrial DNA, genomic DNA or a nucleic acid derived by template-directed synthesis thereof) thus allowing elimination of binding of poly-A or poly-T nucleic acids in the sample to complementary sequences in the capture probe second segment and in the immobilized probe.

[0030] Captured target nucleic acids are useful in a number of analytical techniques, including among others, amplification assays (e.g., the PROCLEIX HIV-1/HCV Assay), base composition determination by mass spectrometry, microarray identification and nucleic acid sequencing assays. For each of these analytical techniques, the nucleic acids entering the assay have a direct impact on the information derived therefrom. The presence of non-target nucleic acids in an assay provides misinformation into the system that then complicates or even makes impossible a proper interpretation of the resulting data. Misinformation includes false positive signals, loss of robustness and sensitivity in the assay, and ambiguous results. Target nucleic acids captured using the compositions and methods of the current invention are more suitable for subsequent analyses compared to nucleic acids isolated by prior methods.

II. L-Nucleic Acid

[0031] Nucleic acids existing in nature are D-nucleic acids formed from D-nucleotides. An L-nucleic acid is the enantiomeric form of a D-nucleic acid. The source of stereoisomerism in a nucleic acid resides in the sugar moiety of each monomeric unit forming the nucleic acid (as illustrated in Fig. 1). Except for the stereoisomerisms at the sugar moiety of each monomeric unit, D and L-nucleic acids and their monomeric units are closely analogous. Thus, for example, the sugar moieties of an L-nucleic acid can be linked to the same nucleobases (i.e., adenine, guanine, cytosine, thymine and uracil) as occur in natural DNA or RNA, or any of the many known analogs of these nucleobases. The sugar moiety of L-nucleic acids can be ribose or deoxyribose or similar compounds (e.g., with 2'-methoxy or 2'halide substitutions). The sugar moieties can be linked by sugar phosphodiester linkages as in D-nucleic acids or by any of the analog linkages that have been used with D-nucleic acids, such as phophorothioate or methylphosphonate linkages or peptide-nucleic acid linkages.

[0032] L-nucleotides incorporating at least the conventional nucleobases (i.e., A, C, G, T and U) are commercially available in the phosphoramidite form suitable for solid phase synthesis (e.g., ChemGenes Corporation (Wilmington, USA). L-nucleic acids can be synthesized from L-nucleotides using the same solid phase synthesis procedures as are used for D-nucleic acids (e.g., an ABI synthesizer and standard synthesis protocols). L-nucleotides can also be linked to D-nucleotides by a conventional coupling cycle (see Hauser et al., Nucleic Acids Research, 2006, Vol. 34, No. 18 5101–5111 (2006), thus permitting synthesis of a chimeric nucleic acid having one segment in D-nucleic acid form and the other in L-nucleic form.

[0033] L-nucleic acids hybridize to one another according to analogous principles to D-nucleic acids (e.g., by formation of Watson-Crick or Hoogstein bonds) and have similar stability to hybrids of D-nucleic acids. The duplex formed from L-nucleic acids is a left-handed helix whereas that formed from D-nucleic acids is a right handed helix. Although L-nucleic acids can hybridize to each other, as further illustrated by the Examples, L-nucleic acids and particularly polyA or polyT L-nucleic acids have no ability to hybridize to a complementary segment of a poly A or polyT D-nucleic acid.

III. Capture Probes

[0034] The invention employs chimeric target capture probes having at least first and second segments. The first segment binds to a target nucleic acid either specifically or nonspecifically (see US 6,110,678 and WO 2008/016988). The second segment, sometimes known as a tail, binds to an immobilized probe and thus serves to capture the target nucleic bound to the capture probe to a support linked to an immobilized probe. Capture probes are typically provided in single-stranded form, or if not, are denatured to single-stranded form before or during use (see WO 2006/007567 and US 2009-0286249).

[0035] The first segment of the chimeric capture probe is typically designed to bind to a target nucleic acid sequence of interest. In some capture probes, the first segment is designed to bind to a segment within a particular target nucleic acid and not to (or at least with substantially reduced affinity) to other nucleic acids lacking this segment that are present in the sample. In other capture probes, the first segment is designed to bind to a class of target nucleic acids (e.g., any DNA molecule) and does not necessarily substantially discriminate between individual target nucleic acids within the class (e.g., by use of a randomized sequence).

[0036] For the first segment to bind to a particular target nucleic acid sequence of interest, the first segment can be designed to include a D-nucleic acid that is substantially and preferably exactly complementary to a corresponding segment of the target nucleic acid. The D-nucleic acid of such a first segment preferably includes at least 6, 10, 15 or 20 nucleobase units (e.g., nucleotides). For example, the D-nucleic acid can contain 10-50, 10-40, 10-30 or 15-25 nucleobase units (e.g., D-nucleotides) complementary to corresponding D-nucleotides in the target nucleic acid. Here, as elsewhere in the application, ranges for contiguous nucleic acid sequences are fully inclusive of all whole numbers defining or within the range (10, 11, 12, 13 … 47, 48, 49, 50).

[0037] For the first segment to bind nonspecifically to nucleic acids without necessarily substantially discriminating between different sequences within a class, the first segment can include a random polymer sequence made up of all four standard DNA bases (guanine (G), cytosine (C), adenine (A) and thymine (T)) or all four standard RNA bases (G, C, A, and uracil (U)) (see US 2008/0286775) The random sequence can also include one or more base analogs (e.g., inosine, 5-nitroindole) or abasic positions in the random polymer sequence. Such a random polymer sequence can contain one or more sequences of poly-(k) bases, i.e., a random mixture of G and U or T bases (e.g., see Table 1 of WIPO Handbook on Industrial Property Information and Documentation, Standard ST.25 (1998)). Sequences that include G and U/T bases can be chosen for their "wobble" property, i.e., U/T binds G or A, whereas G binds C or U/T. A capture probe having a first segment synthesized with a random polymer sequence is in fact a finite population of oligonucleotides that contain different random polymer sequences made up of the bases included during the synthesis of the random portion. For example, a population of nonspecific capture probes that include a 15 nt random polymer sequence made up of G, C, A and T consists of 4^{15} members. The first segment can be designed to bind to DNA sequences preferentially relative to RNA or vice versa (see US 2008-0286775).

[0038] The second segment is designed to bind to an immobilized probe. The second segment includes an L-nucleic acid that is substantially and preferably exactly complementary to an L-nucleic acid present in the immobilized probes. The L-nucleic acid of the capture probe preferably includes at least six nucleobase units (e.g., L-nucleotides) and preferably 10-50, 10-40, 10-10 or 15-25 nucleobase units (e.g., L-nucleotides). Ranges for contiguous nucleic acid sequences are fully inclusive of all whole numbers (10, 11, 12, 13 ... 47, 48, 49, 50) defining or within the range. The L-nucleic acid of the capture probe is preferably a homopolymer or combination of two homopolymers in tandem, and more preferably polyA and/or polyT (e.g. $(T)_{0-5}/(A)_{10-40}$, ranges being inclusive of all whole numbers defining or within the range). The term "poly-A" is used interchangeably with homopolymer of adenine nucleobase units, or sometimes simply a homopolymer of adenines. Likewise the term "poly-T" is used interchangeably with homopolymer of thymine nucleobase units or sometimes simply a homopolymer of thymines. A preferred L-nucleic acid is or includes a homopolymer of 30 adenines. The length of the L-nucleic acid (i.e., number of nucleobase units) in the capture probe may or may not be the same as the length of the L-nucleic acid in the immobilized probe.

[0039] The melting temperature of the duplex formed between the L-nucleic acid of the capture probe and L-nucleic acid of the immobilized probe preferably has a lower melting temperature than the duplex formed between the D-nucleic acid of the first segment of the capture probe and the target nucleic acid. The melting temperatures of both duplexes can be calculated by conventional equations relating base composition and length of a duplex to its melting temperature as discussed above. Selection of polyA or polyT homopolymers for the L-nucleic acids of the capture and immobilized probes tends to confer a lower melting temperature than that for a duplex formed between the first segment of the capture probe and the target nucleic acid because the latter duplex usually also contains some C-G pairings, which confer greater stability on a duplex than A-T pairings. A lower melting temperature of the duplex formed between the second segment of the capture probe and the immobilized probe than the duplex formed between the first segment of the capture probe and the target nucleic acid is advantageous in allowing the hybridization to be performed under conditions of higher stringency in which the capture probe first hybridizes to the target nucleic acid and lower stringency in which the capture probe now hybridizes to the target nucleic acid hybridizes to the immobilized probe. When performed in this order, both capture probe and target nucleic acid are in solution when they hybridize in which conditions, hybridization takes place with much faster kinetics.

[0040] The capture probe may or may not include additional segments as well as the first and second segments mentioned above. For example, the D-nucleobase units of the first segment and L-nucleobase units of the second segment can be directly connected by a phosphodiester bond (or any of the analogs thereof discussed above) or can be separated by a short spacer or linker region, which may include nucleotides, or other molecules, such as PEG typically found in linkers. For example, if the second segment is a polyA homopolymer, the first and second segments can be connected by one or more (e.g., three) thymine residues (which can be L or D enantiomers). In some probes, the first and second segments are connected by 0-5 thymine residues. A capture probes can also include a third segment such that the first segment is flanked by the second and third segments. In such an arrangement, the third segment can include an L-nucleic acid complementary to the L-nucleic acid in the second segment, such that the capture probe is capable of self-annealing to form a stem-loop structure in which the second and third segments are annealed as a stem and the first segment forms a loop in between. Such a stem loop structure can only form when the first target nucleic acid is not hybridized with its target nucleic acid. Such an arrangement can be useful in reducing the ability of a capture probe to hybridize with an immobilized probe before the capture probe has bound to its target nucleic acid and in reducing competition between unhybridized capture probe and a detection probe used to detect the target nucleic acid (see US 20060068417).

[0041] Multiple different capture probes can be used in combination in the same reaction. In this case, the different capture probes typically have different first segments complementary to different target nucleic acids or different segments within the same target nucleic acid, and the identical second segments, so they can bind immobilized probes having the complementary sequences to these second segments. Use of multiple different capture probes can be useful in capturing a population of related target sequences that may be present in a sample, for example, sequence and/or length variants. The number of different capture probes can be at least 1, 2, 5, 10, 20, 50 or 100, for example, 1-100 or 2-50 or 3-25, inclusive of all whole numbers defining or within the range.

IV. Immobilized Probe

[0042] An immobilized probe includes an L-nucleic acid joined directly or indirectly to a support. As indicated in the description of the capture probe, the L-nucleic acid is substantially or preferably exactly complementary to an L-nucleic acid in the capture probe, although may or may not be the same length (number of nucleobase units) as the L-nucleic acid in the capture probe. The L-nucleic acid in the immobilized probe preferably contains at least six contiguous L-nucleobase units (e.g., L-nucleotides) and can contain for example 10-45 or 10-40 or 10-30 or 10-25 or 15-25, inclusively, L-nucleobase units, any range being inclusive of all whole numbers defining or within the range. The L-nucleic acid is preferably a homopolymer, and more preferably a homopolymer of adenine or thymine. A preferred form of immobilized probe is or includes a homopolymer of 14 thymine residues for use in combination with a capture probe including a second segment with a homopolymer of adenine residues. The nucleic acid moiety of an immobilized probe is typically provided in single-stranded form, or if not, is denatured to single-stranded form before or during use.

[0043] Any of a variety of materials may be used as a support for the immobilized probes, e.g., matrices or particles made of nitrocellulose, nylon, glass, polyacrylate, mixed polymers, polystyrene, silane polypropylene, and magnetically attractable materials. Monodisperse magnetic spheres are a preferred support because they are relatively uniform in size and readily retrieved from solution by applying a magnetic force to the reaction container, preferably in an automated system. An immobilized probe may be linked directly to the capture support, e.g., by using any of a variety of covalent linkages, chelation, or ionic interaction, or may be linked indirectly via one or more linkers joined to the support. The linker can include one or more nucleotides of either D or L-enantiomeric forms not intended to hybridize to the capture probe but to act as a spacer between the L-nucleic acid of the immobilized probe and its support.

V. Target Nucleic Acid

[0044] A target nucleic acid refers to a nucleic acid molecule or class of nucleic acid molecules that is or may be present within a sample. A target nucleic acid may be a particular type of nucleic acid (e.g., a particular mRNA present in a heterogeneous mixture of mRNA) or can represent a class of molecules (e.g., any DNA or any mRNA present in a sample). A target nucleic acid includes a segment (target segment) that hybridizes with the first segment on the capture probe to form a stable duplex between D-nucleic acids. The target segment can be the same or substantially the same length as the D-nucleic acid of the first segment of the capture probe and exactly or substantially complementarity to this nucleic acid. The target segment can be only a small fraction of the total length of a target nucleic acid. For example, a target nucleic acid can be several thousand nucleotides long and a target segment can be for example, only 10-30 of these nucleotides. A target nucleic acid can exist in different forms, i.e., single-stranded, double-stranded, triple-stranded, or mixtures thereof, such as in a partially double-stranded hairpin structure or partially double-stranded duplex structure, and a target segment can present on any strand (sense or anti-sense) of the structure. A target nucleic acid can be RNA (e.g., viral RNA, micro RNA, mRNA, cRNA, rRNA, hnRNA or DNA (genomic or cDNA) among others. The target nucleic acid can be from a pathogenic microorganism, such as a virus, bacteria or fungus, or can be endogenous to a patient. A target nucleic acid can be synthetic or naturally occurring. A target nucleic acid can range in length from at least about ten nucleotides to more than 1000 nucleotides or up to 10,000 nucleotides or even greater than 10,000 nucleotides. Target nucleic acids having 25-10,000 nucleotides are common.

[0045] Viral nucleic acids (e.g., genomic, mRNA or cDNA) form a useful target for detection or analyses of viruses. Nucleic acid based detection is particularly useful soon after infection, when transcription and production of viral proteins and antibodies may not have occurred to a detectable extent for an immunoassay. Some examples of viruses that can be detected include HIV, hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, CMV, and Epstein Barr virus), adenovirus, XMRV, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, cornovirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, MLV-related Virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

[0046] Analysis of viral nucleic acids is also useful for analyzing drug resistance. Viruses mutate rapidly so that a patient is often infected with a heterogeneous population of viral nucleic acids, which changes over time. Deconvolution of the population to detect individual variants allows detection of drug resistant mutations and their change over time, thus allowing treatment regimes to be customized to take into account the drug resistance of strains infecting a particular patient.

[0047] Human nucleic acids are useful for diagnosing diseases or susceptibility towards disease (e.g., cancer gene fusions, BRACA-1 or BRAC-2, p53, CFTR, cytochromes P450), for genotyping (e.g., forensic identification, paternity testing, heterozygous carrier of a gene that acts when homozygous, HLA typing), determining drug efficacy on an individual (e.g., companion diagnostics) and other uses.

[0048] rRNA is particularly useful for detecting and/or typing pathogenic bacteria. Examples of such bacteria include chlamydia, rickettsial bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, Lymes disease bacteria, streptococci, or neisseria.

VI. Sample

[0049] A "sample" or "biological sample" refers to any composition or mixture in which a target nucleic acid of interest may be present, including plant or animal materials, waste materials, materials for forensic analysis, environmental samples, and the like. A biological sample includes any tissue, cell, or extract derived from a living or dead organism which may contain a target nucleic acid, e.g., peripheral blood, bone marrow, plasma, serum, biopsy tissue including lymph nodes, respiratory tissue or exudates, gastrointestinal tissue, urine, feces, semen, or other body fluids. Samples of particular interest are tissue samples (including body fluids) from a human or an animal having or suspected of having a disease or condition. Other samples of interest include industrial samples, such as for water testing, food testing, contamination control, and the like.

[0050] Sample components may include target and non-target nucleic acids, and other materials such as salts, acids, bases, detergents, proteins, carbohydrates, lipids and other organic or inorganic materials.

[0051] A sample may or may not be subject of processing to purify or amplify a target nucleic acid before performing the target capture assay described below. It is not, for example, necessary to perform a column binding of elution of nucleic acids. Such a step concentrates and purifies nucleic acids but also can lose a large proportion of the sample. Further processing can include simple dilution of a biological fluid with a lysing solution to more complex methods that are well known in the art (e.g., Su et al., J. Mol. Diagn. 2004, 6:101-107; Sambrook, J. et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd ed., pp. 7.37-7.57; and U.S. Pat. Nos.

5,374,522, 5,386,024, 5,786,208, 5,837,452, and 6,551,778). Typically, a sample containing a target nucleic acid is heated to inactivate enzymes in the sample and to make the nucleic acids in the sample single-stranded (e.g., 90-100°C for 2-10 min, then rapidly cooling to 0-5°C).

VII. Target Capture Assay

[0052] A target capture assay is performed using one or more chimeric capture probes of the invention, an immobilized probe, a sample and a suitable medium to permit hybridization of the capture probe to the target nucleic acid and of capture probe to the immobilized probe. Usually, the target sample is heated before performing the assay to denature any nucleic acids in double-stranded form. The components can be mixed in any order. For example the capture probe can be added to the sample and hybridized with the target nucleic acid in the sample before adding the immobilized probe. Alternatively, the capture probe can already be hybridized to the immobilized probe before supplying these two probes to the assay mix. However, for an automated assay, it is preferable to minimize the number of adding steps by supplying the capture probe and immobilized probe at the same or substantially the same time. In this case, the order of hybridization can be controlled by performing a first hybridization under conditions in which a duplex can form between the capture probe and the target nucleic acid but which exceeds the melting temperature of the duplex that would form between the capture probe and immobilized probe, and then performing a second hybridization under conditions of reduced stringency. Stringency can most easily be reduced by lowering the temperature of the assay mix.

[0053] Following formation of the target nucleic acid:capture probe: immobilized probe hybrid (the capture hybrid complex) can be isolated away from other sample components by physically separating the capture support using any of a variety of known methods, e.g., centrifugation, filtration, or magnetic attraction of a magnetic capture support. To further facilitate isolation of the target nucleic acid from other sample components that adhere non-specifically to any portion of the capture hybrid, the capture hybrid may be washed one or more times to dilute and remove other sample components. Washing may be accomplished by dissociating the capture hybrid into its individual components in an appropriate aqueous solution (e.g., a solution containing Tris and EDTA. See e.g., US Pat. No. 6,110,678) and appropriate conditions (e.g., temperature above the Tm of the components) and then readjusting the conditions to permit reformation of the capture hybrid. However, for ease of handling and minimization of steps, washing preferably rinses the intact capture hybrid attached to the capture support in a solution by using conditions that maintain the capture hybrid.

[0054] Next the target nucleic acid can be released from the capture hybrid, although amplification and/or detection can alternatively be performed while the target nucleic acid is still bound to the capture probe. Release of the target or capture hybrid components can be performed by several methods, such as, e.g., changing one or more conditions to promote dissociation of components (e.g., heating above Tm, changing salt concentrations, adding denaturants or competitive binding moieties to the mixture, or by including compounds in the reaction that improve hybridization, such as imidazole compounds), or by using other conventional methods such as strand displacement. Typically, a simple heating step is performed to melt the target and capture probe strands, e.g., in an aqueous solution of low ionic strength, at 90-100°C for 5 min, followed by rapid cooling to 0-5°C. Other components of the capture hybrid may be released (e.g., capture probe), but only the target nucleic acid must be made available to bind to the detection probe. The soluble phase containing the released target nucleic acid may be separated from other components of the mixture (e.g., capture support and/or unbound capture probes) but this is not critical because the capture probe strand is of the same sense as the detection probe and, therefore, will not interfere with the detection probe binding to the target.

[0055] Preferably, capture of the target nucleic acid with washing if performed, removes at least 70%, preferably at least 90%, and more preferably about 95% of the target nucleic acids from other sample components.

[0056] The target nucleic acid can optionally be subject to a reverse transcription or amplification step, which can be performed with or without release of the target nucleic acid from the capture complex. PCR amplification is described in PCR Technology: Principles and Applications for DNA Amplification (ed. H.A. Erlich, Freeman Press, NY, NY, 1992); PCR Protocols: A Guide to Methods and Applications (eds. Innis, et al., Academic Press, San Diego, CA, 1990); Mattila et al., Nucleic Acids Res. 19, 4967 (1991); Eckert et al., PCR Methods and Applications 1, 17 (1991); PCR (eds. McPherson et al., IRL Press, Oxford); and U.S. Patent 4,683,202. Other suitable amplification methods include the ligase chain reaction (LCR) (see Wu and Wallace, Genomics 4, 560 (1989), Landegren et al., Science 241, 1077 (1988), transcription amplification (Kwoh et al., Proc. Natl. Acad. Sci. USA 86, 1173 (1989)), and selfsustained sequence replication (Guatelli et al., Proc. Nat. Acad. Sci. USA, 87, 1874 (1990)) and nucleic acid based sequence amplification (NASBA).

[0057] A detection step can be performed in soluble phase by adding a detection probe directly to the soluble phase containing the released target nucleic acid and incubating the mixture in hybridization conditions suitable for binding the detection probe and target sequences (e.g., adding salts to the soluble phase to make a solution of suitable ionic strength and incubating at 25-60°C). The detection probe can optionally be designed to be complementary or substantially complementary to the same segment of the target nucleic acid as the capture probe. Although the capture probe is released into solution it will not hybridize to the detection probe because the capture and detection probe oligomers are strands of the same sense. However, the released capture probe can compete with the detection probe for hybridization to the target nucleic acid. Thus, the detection probe is preferably provided in excess or designed to exhibit higher affinity (compared to the capture probe) for the target nucleic acid by virtue of the detection probe's length and/or structural modifications (e.g., backbone). For example, the detection probe can have a longer target-complementary sequence than the capture probe's target-complementary sequence. Optionally, if the capture probe includes a third segment capable of intramolecular duplex formation with the second segment, intramolecular hybridization can form a hairpin form of the capture probe decreasing its ability to compete with a detection probe in subsequent steps. The detection probe can also be designed to bind to a different segment of the same target nucleic acid than the capture probe in which case the capture probe does not compete with the detection probe for binding to the target nucleic acid. After the detection probe binds to the target nucleic acid to form the detection hybrid, a signal from the hybrid is detected to indicate the presence of the target in the tested sample. Optionally, unbound detection probe can be removed before signal detection. However, the detection hybrid is preferably detected in a homogeneous reaction to avoid having to separate the unbound probes before signal detection from the bound probes (e.g., as described in U.S. Pat. Nos. 5,283,174, 5,639,604, 5,948,899, 5,658,737, 5,756,709, 5,827,656, and 5,840,873). Detection of a signal from the detection hybrid indicates the presence of the target nucleic acid in the sample.

[0058] Optionally, one or more additional oligomers may bind to the target nucleic acid in the detection step to facilitate binding the detection probe and/or producing a detectable signal. Such additional oligomers include, e.g., helpers, competitive probes for cross-reacting non-target sequences, or an oligomer that brings another component used in signal production (e.g., enzyme, substrate, catalyst, or energy emitter) into proximity with the detection probe (U.S. Pat. No. 5,030,557, Hogan et al.; U.S. Pat. No. 5,434,047, Arnold; and U.S. Pat. No. 5,928,862, Morrison).

[0059] As well as or instead of performing a simple detection step that may detect the presence and/or amount of a target nucleic acid in a sample, a captured target nucleic acid can also be subject to a more detailed analysis, for example, to detect mutations that may be present in a sample. The capture of target nucleic acid can be coupled to several different formats of so-called next generation and third generation sequencing methods. Such methods can sequence millions of target templates in parallel. Such methods are particularly useful when the target nucleic acid is a heterogeneous mixture of variants, such as is often the case in a sample from a patient infected with a virus, such as HIV. Among the many advantages, sequencing variants in parallel provides a profile of drug resistant mutations in the sample, even drug mutations present in relatively minor proportions within the sample.

[0060] Some next generation sequence methods amplify by emulsion PCR. A target nucleic acid immobilized to beads via a capture probe provides a suitable starting material for emulsion PCR. The beads are mixed with PCR reagents and emulsion oil to create individual micro reactors containing single beads (Margulies et al., Nature 437, 376-80 (2005)). The emulsion is then broken and the individual beads with amplified DNA are sequenced. The sequencing can be pyrosequencing performed for example using a Roche 454 GS FLX sequencer (454 Life Sciences, Branford, CT 06405). Alternatively, sequencing can be ligation/detection performed for example using an ABI SOLiD Sequencing System (Life Technologies, Carlsbad, CA 92008). In another variation, target nucleic acids are eluted from the capture probe and immobilized in different locations on an array (e.g., the HiScanSQ (Illumina, San Diego, CA 92121)). The target nucleic acids are amplified by bridge amplification and sequenced by template-directed incorporation of labeled nucleotides, in an array format (Illumina). In another approach, target nucleic acids are eluted from the capture probe and single molecules are analyzed by detecting in real-time the incorporation nucleotides by a polymerase. The nucleotides can be labeled nucleotides that release a signal when incorporated (e.g., Pacific Biosciences, Eid et al., Sciences 323 pp. 133 – 138 (2009) or unlabeled nucleotides, wherein the system measures a chemical change upon incorporation (e.g., Ion Torrent Personal Genome

Machine (Guilform, CT 94080)). If labeled, different nucleotides can have the same or different labels as each other.

[0061] Although captured target nucleic acids can be sequenced by any technique, third generation, next generation or massively parallel methods offer considerable advantages over traditional techniques. Two traditional techniques for sequencing DNA are the dideoxy termination method of Sanger (Sanger et al., PNAS USA, 74: 5463 (1977)) and the Maxam-Gilbert chemical degradation method (Maxam and Gilbert, PNAS USA, 74: 560 (1977)). Both methods deliver four samples with each sample containing a family of DNA strands in which all strands terminate in the same nucleotide. Ultrathin slab gel electrophoresis, or more recently capillary array electrophoresis is used to resolve the different length strands and to determine the nucleotide sequence, either by differentially tagging the strands of each sample before electrophoresis to indicate the terminal nucleotide, or by running the samples in different lanes of the gel or in different capillaries. Both the Sanger and the Maxam-Gilbert methods are labor- and time-intensive, and require extensive pretreatment of the DNA source.

[0062] The concept of sequencing DNA by synthesis without using electrophoresis was first revealed in 1988 (Hyman, Analytical Biochemistry, 174: 423 (1988)) and involves detecting the identity of each nucleotide as it is incorporated into the growing strand of DNA in polymerase reaction. Such a scheme coupled with the chip format and laser-induced fluorescent detection markedly increases the throughput of DNA sequencing projects. Several groups have described such systems with an aim to construct an ultra high-throughput DNA sequencing procedure (see. e.g., Cheeseman, US Pat. No. 5,302,509, Metzker et al., Nucleic Acids Res. 22: 4259 (1994)). The pyrosequencing approach that employs four natural nucleotides (comprising a base of adenine (A), cytosine (C), guanine (G), or thymine (T)) and several other enzymes for sequencing DNA by synthesis is now widely used for mutation detection (Ronaghi, Science 281, 363 (1998); Binladin et al., PLoS ONE, issue 2, e197 (February 2007); Rehman et al., American Journal of Human Genetics, 86, 378 (March 2010); Lind et al, Human Immunology 71, 1033-1042 (2010); Shafer et al., J Infect Dis. 1;199(5):610 (2009)). In this approach, the detection is based on the pyrophosphate (PPi) released during the DNA polymerase reaction, the quantitative conversion of pyrophosphate to adenosine triphosphate (ATP) by sulfurylase, and the subsequent production of visible light by firefly luciferase. DNA sequences can be deduced by measuring pyrophosphate release on testing DNA/polymerase complexes with each deoxyribonucleotide triphosphate (dNTP) separately and sequentially. See Ronaghi et al., Science 281: 363 365 (1998); Hyman, Anal. Biochem. 174, 423 (1988); Harris, US Pat. No. 7,767,400. Alternatively, DNA sequencing can be performed by a synthesis method mostly focused on a photocleavable chemical moiety that is linked to a fluorescent dye to cap the 3'-OH group of deoxynucleoside triphosphates (dNTPs) (Welch et al. Nucleosides and Nucleotides 18, 197 (1999) & European Journal, 5:951-960 (1999); Xu et al., US Pat. No. 7,777,013; Williams et al., US Pat. No. 7,645,596; Kao et al, US Pat. No. 6,399,335; Nelson et al., US Pat. Nos. 7,052,839 & 7,033,762; Kumar et al., US Pat. No. 7,041,812; Sood et al, US Pat. App. No. 2004-0152119; Eid et al., Science 323, 133 (2009)).

[0063] Sequencing platforms are further moving away from those that read a plurality of target nucleic acids towards single molecule sequencing systems. Earlier systems analyze target nucleic acids in bulk. What this means is that, for example with Sanger sequencing, a plurality of target nucleic acids are amplified in the presence of terminating ddNTPs. Collectively, each termination position read on a gel represents a plurality of amplification products that all terminated at the same nucleobase position. Single-molecule sequencing systems can use nanostructures wherein the synthesis of a complementary strand of nucleic acid from a single template is performed. These nanostructures are typically configured to perform reads of a plurality of single strand nucleic acids. Each single strand contributes sequence information to the sequence analysis system. See, Hardin et al., 7,329,492; Odera, US Pub. Pat. App No. 2003-0190647. Single-molecule sequence is preferably performed in real-time, meaning that a nucleobase unit is detected before incorporation of the next nucleobase unit, as is the case for the Pacific Biosciences method mentioned above.

[0064] Sequencing technologies are known in the art. For a further review of some sequencing technologies, see Cheng, Biochem. Biophys. 22: 223 227 (1995); Mardis, Annual Review of Genomics and Human Genetics 9: 387–402 (2008) & Genome Medicine 1 (4): 40 (2009); Eid et al., Science 323, 133 (2009); Craighead et al., US Pat. No. 7,316,796; Lipshutz, et al., Curr Opinion in Structural Biology., 4:376 (1994); Kapranov et al., Science 296, 916 (2002); Levene et al., US Pat. No. 6,917,726, Korlach et al., US Pat. No. 7,056,661; Levene et al. Science 299, 682 (2003); Flusberg et al., Nature Methods v.7, no.6, p.461 (June 2010); Macevicz, US Pat. Nos. 6,306,597 & 7,598,065; Balasubramanian et al., US Pat. No. 7,232,656; Lapidus et al, US Pat. No. 7,169,560; Rosenthal et al., US Pat. No. 6,087,095; Lasken, Curr Opin Microbiol.

10(5):510 (2007); Ronaghi et al., Pharmacogenics. Volume 8, 1437-41 (2007); Keating et al., PLoS One 3(10):e3583 (2008); Pease et al., PNAS USA 91(11):5022 (1994); Lockhart, et al., Nat Biotechnol. 14(13):1675 (1996); Shendure et al., Science 309, 1728 (2005); Kim et al., Science 316, 1481 (2007); Valouev et al. Genome Research 18 (7): 1051 (2008); Cloonan et al., Nature Methods 5 (7): 613 (2008); Tang et al. Nature Methods 6 (5): 377 (2009); McKernan et al. Genome Research 19 (9): 1527 (2009); Ecker et al., Nature Reviews Microbiology 6, 553 (2008).

VI. Kits

[0065] The invention also provides kits for performing the methods for detecting target nucleic acids described herein. Preferred kits contain at least one capture probe and at least one immobilized probe as described above. Kits can also include a detection probe specific for a target nucleic acid. In preferred kits, the immobilized probe is immobilized to a magnetized particle, preferably a paramagnetic bead, with homopolymeric oligomers (e.g., polyA, polyT, polyC, or polyG) attached to it that are complementary to a homopolymeric portion of the capture probe in the kit. Kit can also include chemical compounds used in forming the capture hybrid and/or detection hybrid, such as salts, buffers, chelating agents, and other inorganic or organic compounds. Kit can also include chemical compounds used in releasing the target nucleic acid from a capture hybrid, such as salts, buffers, chelating agents, denaturants, and other inorganic or organic compounds. Kit can also include chemical compounds used in the detection step, such as enzymes, substrates, acids or bases to adjust pH of a mixture, salts, buffers, chelating agents, and other inorganic or organic compounds used in producing a detectable signal from a detection hybrid. Kit can also include chemicals for preparing samples for use in the invention methods which may include individual components or mixtures of lysing agents for disrupting tissue or cellular material and preserving the integrity of nucleic acids. Such compositions include enzymes, detergents, chaotropic agents, chelating agents, salts, buffering agents, and other inorganic or organic compounds. Kits can include any combination of the capture probe, detection probe, and immobilize probe components described above which can be packaged in combination with each other, either as a mixture or in individual containers. Kits can also contain instructions for performing the capture methods described above.

[0066] Although the invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims. To the extent difference sequences might be associated with the same accession number at different times, the sequence associated with the accession number at the effective filing date is meant. The effective filing date means the earliest priority date at which the accession number at issue is disclosed. Unless otherwise apparent from the context any element, embodiment, step, feature or aspect of the invention can be performed in combination with any other

EXAMPLES

Example 1

[0067] The purpose of this experiment was to demonstrate that a homopolymer of 14 units L-thymine deoxyribose (T14) hybridizes only to an L-adenine deoxyribose polymer (A30) not to the natural (D) adenine deoxyribose polymer (A30). Magnetic particles were prepared with covalently attached immobilized probes comprising 5'-TTTTTTTTTTTTTT-3' in the left handed form for one set and in the right handed form for another set. Left and right handed chimeric capture probed having the sequence 5'-CUCUUCCAAUCGUCCGCGUGCUUATTTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3' were also prepared (underlined portion of the sequence represents the nucleic acids that were made in separate sets of left-handed form or right handed form). The target was a ribosomal RNA nucleic acid and capture was determined using an AE labeled nucleic acid probe having the sequence 5'-CUCCUAUCGUUCCAUAGUCACCCT-3'.

The target ribosomal RNA has a nucleic acid of:

TTTTTTCTGAGAATTTGATCTTGGTTCAGATTGAACGCTGGCGGCGTGGATGAGGCA
TGCAAGTCGAACGGAGCAATTGTTTCGGCAATTGTTTAGTGGCGGAAGGGTTAGTAA
TGCATAGATAATTTGTCCTTAACTTGGGAATAACGGTTGGAAACGGCCGCTAATACC
GAATGTGGCGATATTTGGGCATCCGAGTAACGTTAAAGAAGGGGATCTTAGGACCT
TTCGGTTAAGGGAGAGTCTATGTGATATCAGCTAGTTGGTGGGGTAAAGGCCTACCA
AGGCTATGACGTCTAGGCGGATTGAGAGATTGGCCGCCAACACTGGGACTGAGACA
CTGCCCAGACTCCTACGGGAGGCTGCAGTCGAGAATCTTTCGCAATGGACGGAAGT
CTGACGAAGCGACGCCGCGTGTGTGATGAAGGCTCTAGGGTTGTAAAGCACTTTCGC
TTGGGAATAAGAGAAGGCGGTTAATACCCGCTGGATTTGAGCGTACCAGGTAAAGA
AGCACCGGCTAACTCCGTGCCAGCAGCTGCGGTAATACGGAGGGTGCTAGCGTTAA
TCGGATTTATTGGGCGTAAAGGGCGTGTAGGCGGAAAGGTAAGTTAGTTGTCAAAG
ATCGGGGCTCAACCCCGAGTCGGCATCTAATACTATTTTTCTAGAGGGTAGATGGAG
AAAAGGGAATTTCACGTGTAGCGGTGAAATGCGTAGATATGTGGAAGAACACCAGT

GGCGAAGGCGCTTTTCTAATTTATACCTGACGCTAAGGCGCGAAAGCAAGGGGAGC
AAACAGGATTAGATACCCTGGTAGTCCTTGCCGTAAACGATGCATACTTGATGTGGA
TGGTCTCAACCCCATCCGTGTCGGAGCTAACGCGTTAAGTATGCCGCCTGAGGAGTA
CACTCGCAAGGGTGAAACTCAAAAGAATTGACGGGGGCCCGCACAAGCAGTGGAG
CATGTGGTTTAATTCGATGCAACGCGAAGGACCTTACCTGGGTTTGACATGTATATG
ACCGCGGCAGAAATGTCGTTTTCCGCAAGGACATATACACAGGTGCTGCATGGCTGT
CGTCAGCTCGTGCCGTGAGGTGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATC
GTTAGTTGCCAGCACTTAGGGTGGGAACTCTAACGAGACTGCCTGGGTTAACCAGG
AGGAAGGCGAGGATGACGTCAAGTCAGCATGGCCCTTATGCCCAGGGCGACACACG
TGCTACAATGGCCAGTACAGAAGGTAGCAAGATCGTGAGATGGAGCAAATCCTCAA
AGCTGGCCCCAGTTCGGATTGTAGTCTGCAACTCGACTACATGAAGTCGGAATTGCT
AGTAATGGCGTGTCAGCCATAACGCCGTGAATACGTTCCCGGGCCTTGTACACACCG
CCCGTCACATCATGGGAGTTGGTTTTACCTTAAGTCGTTGACTCAACCCGCAAGGGA
GAGAGGCGCCCAAGGTGAGGCTGATGACTAGGATGAAGTCGTAACAAGGTAGCCCT
ACCGGAAGGTGGGGCTGGATCACCTCCTTTTAAGGATAAGGAAGAAGCCTGAGAAG
GTTTCTGACTAGGTTGGGCAAGCATTTATATGTAAGAGCAAGCATTCTATTTCATTTG
TGTTGTTAAGAGTAGCGTGGTGAGGACGAGACATATAGTTTGTGATCAAGTATGTTA
TTGTAAAGAAATAATCATGGTAACAAGTATATTTCACGCATAATAATAGACGTTTAA
GAGTATTTGTCTTTTAGGTGAAGTGCTTGCATGGATCTATAGAAATTACAGACCAAG
TTAATAAGAGCTATTGGTGGATGCCTTGGCATTGACAGGCGAAGAAGGACGCGAAT
ACCTGCGAAAAGCTCCGGCGAGCTGGTGATAAGCAAAGACCCGGAGGTATCCGAAT
GGGGAAACCCGGTAGAGTAATAGACTACCATTGCATGCTGAATACATAGGTATGCA
GAGCGACACCTGCCGAACTGAAACATCTTAGTAGGCAGAGGAAAAGAAATCGAAG
AGATTCCCTGTGTAGCGGCGAGCGAAAGGGGAATAGCCTAAACCGAGCTGATAAGG
CTCGGGGTTGTAGGATTGAGGATAAAGGATCAGGACTCCTAGTTGAACACATCTGG
AAAGATGGATGATACAGGGTGATAGTCCCGTAGACGAAAGGAGAGAAAGACCGAC
CTCAACACCTGAGTAGGACTAGACACGTGAAACCTAGTCTGAATCTGGGGAGACCA
CTCTCCAAGGCTAAATACTAGTCAATGACCGATAGTGAACCAGTACTGTGAAGGAA
AGGCGAAAAGAACCCTTGTTAAGGGAGTGAAATAGAACCTGAAACCAGTAGCTTAC
AAGCGGTCGGAGACCAATGGCCCGTAAGGGTCAAGGTTGACGGCGTGCCTTTTGCA
TGATGAGCCAGGGAGTTAAGCTAAACGGCGAGGTTAAGGGATATACATTCCGGAGC
CGGAGCGAAAGCGAGTTTTAAAAGAGCGAAGAGTCGTTTGGTTTAGACACGAAACC
AAGTGAGCTATTTATGACCAGGTTGAAGCATGGGTAAAACTATGTGGAGGACCGAA
CTAGTACCTGTTGAAAAAGGTTTGGATGAGTTGTGAATAGGGGTGAAAGGCCAATC
AAACTTGGAGATATCTTGTTCTCTCCGAAATAACTTTAGGGTTAGCCTCGGATAATG
AGCTTTTGGGGGTAGAGCACTGAATTCTAGCGGGGGCCTACCGGCTTACCAACGGA
AATCAAACTCCGAATACCAGAAGCGAGTCCGGGAGATAGACAGCGGGGGCTAAGCT
TCGTTGTCGAGAGGGGAACAGCCCAGACCGCCGATTAAGGTCCCTAATTTTATGCTA
AGTGGGTAAGGAAGTGATGATTCGAAGACAGTTGGAATGTTGGCTTAGAGGCAGCA
ATCATTTAAAGAGTGCGTAACAGCTCACCAATCGAGAATCATTGCGCCGATAATAA
ACGGGACTAAGCATAAAACCGACATCGCGGGTGTGTCGATAAGACACGCGGTAGGA
GAGCGTAGTATTCAGCAGAGAAGGTGTACCGGAAGGAGGGCTGGAGCGGATACTAG
TGAAGATCCATGGCATAAGTAACGATAAAGGGAGTGAAAATCTCCCTCGCCGTAAG
CCCAAGGTTTCCAGGGTCAAGCTCGTCTTCCCTGGGTTAGTCGGCCCCTAAGTTGAG
GCGTAACTGCGTAGACGATGGAGCAGCAGGTTAAATATTCCTGCACCACCTAAAAC
TATAGCGAAGGAATGACGGAGTAAGTTAAGCACGCGGACGATTGGAAGAGTCCGTA

GAGCGATGAGAACGGTTAGTAGGCAAATCCGCTAACATAAGATCAGGTCGCGATCA
AGGGGAATCTTCGGAGGAACCGATGGTGTGGAGCGAGGCTTTCAAGAAATAATTTC
TAGCTGTTGATGGTGACCGTACCAAAACCGACACAGGTGGGCGAGATGAATATTCT
AAGGCGCGCGAGATAACTTTCGTTAAGGAACTCGGCAAATTATCCCCGTAACTTCGG
AATAAGGGGAGCCTTTTAGGGTGACTATGGAACGATAGGAGCCCCGGGGGGCCGCA
GAGAAATGGCCCAGGCGACTGTTTAGCAAAAACACAGCACTATGCAAACCTCTAAG
GGGAAGTATATGGTGTGACGCCTGCCCAATGCCAAAAGGTTAAAGGGATATGTCAG
CTGTAAAGCGAAGCATTGAACCTAAGCCCTGGTGAATGGCCGCCGTAACTATAACG
GTGCTAAGGTAGCGAAATTCCTTGTCGGGTAAGTTCCGACCTGCACGAATGGTGTAA
CGATCTGGGCACTGTCTCAACGAAAGACTCGGTGAAATTGTAGTAGCAGTGAAGAT
GCTGTTTACCCGCGAAAGGACGAAAAGACCCCGTGAACCTTTACTGTACTTTGGTAT
TGGTTTTTGGTTTGTTATGTGTAGGATAGCCAGGAGACTAAGAACACTCTTCTTCAG
GAGAGTGGGAGTCAACGTTGAAATACTGGTCTTAACAAGCTGGGAATCTAACATTA
TTCCATGAATCTGGAAGATGGACATTGCCAGACGGGCAGTTTTACTGGGGCGGTATC
CTCCTAAAAAGTAACGGAGGAGCCCAAAGCTTATTTCATCGTGGTTGGCAATCACGA
GTAGAGCGTAAAGGTATAAGATAGGTTGACTGCAAGACCAACAAGTCGAGCAGAGA
CGAAAGTCGGGCTTAGTGATCCGGCGGTGGAAAGTGGAATCGCCGTCGCTTAACGG
ATAAAAGGTACTCCGGGGATAACAGGCTGATCGCCACCAAGAGTTCATATCGACGT
GGCGGTTTGGCACCTCGATGTCGGCTCATCGCATCCTGGGGCTGGAGAAGGTCCCAA
GGGTTTGGCTGTTCGCCAATTAAAGCGGTACGCGAGCTGGGTTCAAAACGTCGTGAG
ACAGTTTGGTCTCTATCCTTCGTGGGCGCAGGATACTTGAGAGGAGCTGTTCCTAGT
ACGAGAGGACCGGAATGGACGAACCAATGGTGTATCGGTTGTTTTGCCAAGAGCAT
AGCCGAGTAGCTACGTTCGGAAAGGATAAGCATTGAAAGCATCTAAATGCCAAGCC
TCCCTCAAGATAAGGTATCCCAATGAGACTCCATGTAGACTACGTGGTTGATAGGTT
GGAGGTGTAAGCACAGTAATGTGTTCAGCTAACCAATACTAATAAGTCCAAAGACT
TGGTCTTTTTATGATTGGAAGAGCCGAAAGGCAAAGACAATAAGAAAAAGAGTAGA
GAGTGCAAGTGCGTAGAAGACAAGCTTTTAAGCGTCTATTAGTATACGTGAGAAAC
GATACCAGGATTAGCTTGGTGATAATAGAGAGAGGA

[0068] The capture and hybridization reactions were performed as substantially described in the art (Pace2 kits (Gen-Probe Incorporated, San Diego, CA 92121); US Pat. No. 6,110,678; WO 2006/007567 & US 2009-0286249). Briefly, the target nucleic acid and the probe were hybridized at 60°C for 1 hour, and then combined with one of four target capture reaction mixtures. The four target capture reaction mixture set-up was as follow: Bead with L-immobilized probe + capture probe with L-second segment; Bead with D-immobilized probe + capture probe with D-second segment; Bead with L-immobilized probe + capture probe with D-second segment; and Bead with D-immobilized probe + capture probe with L-second segment. Controls were (1) beads + target:probe without capture probe, and (2) beads + capture probe without target:probe. The target capture reactions were incubated at about 60°C for 30 minutes and then a room temperature for about 15 minutes. Capture reactions were washed twice with a wash buffer, target nucleic acids were eluted and AE reactions were determined on a luminometer. Table 1 shows the signal (RLU).

TABLE 1

Condition	RLU
No capture probe	1310
No target	256
L-capture probe and L-immobilized probe	59268
D-capture probe and D-immobilized probe	58253
L-capture probe and D-immobilized probe	1973
D-capture probe and L-immobilized probe	1184

[0069] An L-nucleic acid in the capture probe hybridizes to an L-nucleic acid in the immobilized probe to essentially the same extent as a D-nucleic acid in the capture probe hybridizes to a D-nucleic acid in the immobilized probe. A D-nucleic acid capture probe and an L-nucleic acid immobilized probe or vice versa give essentially only a background signal comparable to that when the capture probe is omitted altogether.

Example 2

[0070] The purpose of this experiment was to demonstrate that L-deoxy thymidine hybridizes only to L-deoxy adenosine and not to any other right handed natural nucleic acids. The reaction was prepared substantially as in example 1, but capture probe was provided at 2, 5, 10 and 20 pmoles per reaction. The target capture reaction mixture set-up was as follow: Bead with L-immobilized probe + capture probe with L-second segment (L-L); Bead with D-immobilized probe + capture probe with D-second segment (D-D); Bead with L-immobilized probe + capture probe with D-second segment (L-D); and Bead with D-immobilized probe + capture probe with L-second segment (D-L). Controls were (1) beads + target:probe without capture probe, and (2) beads + capture probe without target:probe. Results are presented in TABLE 2.

TABLE 2

	RLU	L-L (RLU)	D-D (RLU)	L-D (RLU)	D-L (RLU)	L-L % capture	D-D % capture	L-D % capture	D-L % capture
No Capture Probe	807								
No Target	227								
Supernatant	43481								
Supernatant cont.	5465								
2pmoles		145705	138889	697	1797	76.6	73	0.37	0.94
5pmoles		159518	130179	439	1163	83.9	68.5	0.23	0.61
10 pmoles		162542	144574	489	803	86	76	0.25	0.42
20 pmoles		192300	129737	1020	1400	100	68.2	0.53	0.73

[0071] Again left handed nucleic acids and right handed complementary nucleic acids do not hybridize. The L-L capture resulted in about 77-100% recovery, the D-D capture resulted in about 68-76% recovery and the L-D and D-L captures were not above background.

Example 3

[0072] The purpose of this experiment was to compare the Tm of DdA:DdT vs LdA:LdT and to investigate whether LdA:DdT or DdA:LdT have any Tm at all. Each strand was first hybridized at about 50°C for 1 hour followed by a room temperature incubation for 1 hour. The capture probes and immobilized probes were then incubated at increasing temperatures ranging from about 20°C to about 80°C, and absorbance measurements were taken at numerous time intervals. Hybridizations were performed substantially as described in example 1. Absorbance against temperature of hybridization for L-nucleic acids with D-nucleic acids (LdA:dT or dA:LdT); L-nucleic acids with L-nucleic acids; and D-nucleic acids with D-nucleic acids (LdA and LdT and dA and dT) were determined. Hybrids formed from LdA and LdT and dA and dT have the same melting temperature of 52.1°C. LdA:dT or dA:LdT have no detectable melting point and thus no hybridization.

Example 4

[0073] HIV or HCV can be detected using commercial assays. The assay involves three main steps which take place in a single tube: sample preparation; HIV-1 and HCV RNA target amplification by Transcription-Mediated Amplification (TMA) (US 5,399,491); and detection of the amplification products (amplicon) by a Hybridization Protection Assay (HPA) (Arnold et al., Clin Chem. 35:1588-1594 (1989).

[0074] During sample preparation, RNA is isolated from plasma specimens via the use of target capture. Plasma is treated with a detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Capture probes having first segments complementary to conserved regions of HIV-1 or HCV and second segment including L-polyA, are hybridized to the HIV-1 or HCV RNA target, if present, in the test specimen. The hybridized target is then captured onto an immobilized probe of L-poly-T on magnetic microparticles that are separated from plasma in a magnetic field. Wash steps are utilized to remove extraneous plasma components from the reaction tube. Magnetic separation and wash steps are performed.

[0075] Target amplification occurs via a real-time R-TMA version of TMA, which is a transcription-based nucleic acid amplification method that utilizes two enzymes, MMLV reverse transcriptase and T7 RNA polymerase (see e.g., US 2007-0299254 and US 2006-0068380). The reverse transcriptase is used to generate a DNA copy (containing a promoter sequence for T7 RNA polymerase) of the target RNA sequence. T7 RNA polymerase produces multiple copies of RNA amplicon from the DNA copy template.

Example 5

Introduction

[0076] This example illustrates an HIV-1 L-ribose based target capture reagent (L-TCR) and target capture method developed for deep sequencing of the HIV-1 genome using 24 L-ribose Target Capture Oligos (TCO) three of which were also made in D-ribose form. This new reagent/method was shown to capture and amplify inactivated HIV-1 from Procleix HIV-1 Positive Control. Using an elution protocol and adding 1/30th of eluate to the Reverse TMA reaction, 2000 copy/mL inactivated HIV-1 RNA was detected.

Bead Design

[0077] L-TCOs consisted of a terminal dA with the L-ribose beginning at the 29th dA and the target sequence reverting to D-ribose for the dT3 and capture nucleotides.

[0078] Twenty-four TCO sequences were designed. All HIV-1 genome sequences from the 2007 curated alignment of the Los Alamos National Laboratory HIV Database were imported and a consensus alignment from the 1242 sequences was created. The TCOs, ranging from 17-36 nucleotides in length for the specific target region, were designed using the consensus sequence, Walking along the genome, TCOs were spaced from 100–882 nucleotides apart with an average spacing of 481 nucleotides. Table 3 shows the target capture oligomers. The polyA sequence of the target capture oligomer is shown in bold.

TABLE 3

TCO	Sequence (5' → 3')
A	UUUAAGCAGUGGGUUCCCUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
B	GCAGCUGCUUAUAUGCAGCAAUCUGAGGGTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
C	ACUAGCUUGAAGCACCAUCCAAATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
D	UCCAGUCCCCCCUUUUCUUUUAAATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
E	GUAGCUGAAGAGGCACAGGCUCCGTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
F	AAGCCUCCUACUAUCAUUAUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
G	UUUAUAUUUAUAUAAUUCACUUCUCCAAUUGUCCTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
H	GGAGGGGCAUACAUUGCUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
I	UGAGGUAUUACAAUUUAUUAATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
J	UUCUUGUGGGUUGGGGUCUGUGGGUACACAGTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
K	GCCACUGUCUUCUGCUCUUUCTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
L	GUCUCCGCUUCUUCCUGCCAUAGGATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
M	GGUCUUCUGGGGCUUGUUCCAUCUAUCTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
N	UCUUGUAUUACUACUGCCCCUUCACCUUUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
O	CCAUCUUCCCCCUGCUAAUUUUATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
P	ACUAAUUUAUCUACUUGUUCAUUUCCUCCAAUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
Q	GCUAUUAAGUCUUUUGAUGGGUCAUAAUATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
R	UGGAAUAUUGCUGGUGAUCCUUUCCAUCCCUGUGGTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**

TABLE 3

TCO	Sequence (5' → 3')
S	CAUUCCUGGCUUUAAUUUUACUGGUACAGUUUCTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
T	UGCCAAAGAGUGAUUUGAGGGAATTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
U	CCCACUCCCUGACAUGCUGUCAUCAUUUCUUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
V	UUUAAAUCUUGUGGGGUGGCUCCUUCUGAUAAUGCUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
AA	UCUGCUGUCCCUGUAAUAAACCCGTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**
BB	GCUGGAAUAACUUCUGCUUCUAUTTT**AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA**

Target Protocol Development

[0079] Target capture was performed using substantially the compositions and known methods except for the use of L-nucleic acids (see e.g., US 6,110,678, US 2007-0299254 and US 2006-0068380). Sample was mixed at a 1.25:1 ratio with TCR containing about 2 pmoles/reaction of one of the target capture oligomers in Table 3 and about 20-40 micrograms/reaction of magnetic beads with immobilized probes. Thus, the twenty-four separate reactions were prepared. The mixtures were heated at 60°C for 20 minutes, then annealed on the bench for 15 minutes. The beads were washed on the Target Capture Station, which performs magnetic separation / wash, using an oil free wash solution followed by wash solution with 200 uL oil included. Washed beads were resuspended in 75 uL of Amplification Reagent, transferred to a 96-well plate and briefly centrifuged. The reaction plate was heated at 60°C for about 10 to 15 minutes followed by a cool-down to 42°C on-board a Stratagene Real-Time PCR instrument used here for performing Real-Time RNA transcription mediated amplification (RTMA).

[0080] After several minutes of equilibration at 42°C, 25 uL of enzyme reagent containing M-MLV reverse transcriptase and T7 RNA Polymerase was added, and the reaction amplified and analyzed on the Stratagene analyzer.

L-Ribose TCO Evaluations

[0081] The L-ribose TCOs (L-TCO) were individually evaluated for target capture using HIV-1 Positive Control (500 copies/mL inactivated virus) as the target and using the HIV-1

Discriminatory assay for target detection. Each L-TCO was individually spiked at about 1.82 pmol/rxn into 20 ug/rxn magnetic particles and individually tested for target capture Controls included a: (1) "No-TCO" in TCR control; (2) TCO Sequence AA from TABLE 3 with D nucleic acids in the second segment and a D-nucleic acid immobilized probe linked to a magnetic bead; and (3) TCO Sequence AA from TABLE 3 with L-nucleic acids in the second segment and a L-nucleic acid immobilized probe linked to a magnetic bead.

<u>L-TCOs A through L</u>

[0082] All TCOs exhibited capture as evidenced by amplification with the exception of TCO I, which yielded no target Ct (emergence time) or internal control Ct on initial or repeat testing (see Table 4. With the remaining target capture reactions, there was a variability among the individual target capture oligomers, some being substantially better than others. These varied results between the different target capture oligomers may be due to the weaker binding of the first segment to the target nucleic acid, interference from the internal control, competition between the target capture oligomers and the probe oligomers used or a combination thereof. To check for interference of the TCOs with internal control amplification, the TCO sequences were compared for matches to the internal control oligos and torch sequences. There were typically at most 4-5 consecutive base matches that might explain the interference with internal control amplification. Internal control will not be needed in the final L-TCR intended for PCR.

[0083] Summary: L-TCOs with reasonable Cts and ample signal were L-TCOs F, G, J, K, L. L-TCOs F, K, L came the closest to matching performance of the control R-TCO (AA).

TABLE 4

Well	Dye	Bead	Capture Probe	Threshold	CT (dR)	S/N
A1	FAM	RH Bead[1]	R-AA	498.572	23.24	5.347
A2	FAM	RH Bead	R-No TCO	498.572	23.83	3.772
A3	FAM	LH Bead[2]	L-AA	498.572	23.32	4.45
A4	FAM	LH Bead	L-No TCO	498.572	100	0.971
A5	FAM	LH Bead	A	498.572	34.62	1.417
A6	FAM	LH Bead	B	498.572	36.67	1.311
A7	FAM	LH Bead	C	498.572	33.12	1.395
A8	FAM	LH Bead	D	498.572	100	1.269
B1	FAM	RH Bead	R-AA	498.572	23.15	4.49

TABLE 4

Well	Dye	Bead	Capture Probe	Threshold	CT (dR)	S/N
B2	FAM	RH Bead	R-No TCO	498.572	24.66	3.384
B3	FAM	LH Bead	L-AATCO	498.572	22.31	5.752
B4	FAM	LH Bead	L-No TCO	498.572	100	0.981
B5	FAM	LH Bead	A	498.572	32.92	1.498
B6	FAM	LH Bead	B	498.572	100	1.177
B7	FAM	LH Bead	C	498.572	30.74	1.655
B8	FAM	LH Bead	D	498.572	100	1.211
C1	FAM	RH Bead	R-AA	498.572	24.27	4.136
C2	FAM	RH Bead	R-No TCO	498.572	27.48	2.327
C3	FAM	LH Bead	L-AA	498.572	24.72	3.582
C4	FAM	LH Bead	L-No TCO	498.572	100	0.968
C5	FAM	LH Bead	A	498.572	100	1.241
C6	FAM	LH Bead	B	498.572	100	0.979
C7	FAM	LH Bead	C	498.572	27.77	2.291
C8	FAM	LH Bead	D	498.572	34.65	1.484
D1	FAM	LH Bead	E	498.572	100	1.079
D2	FAM	LH Bead	F	498.572	25.41	2.671
D3	FAM	LH Bead	G	498.572	27.29	2.305
D4	FAM	LH Bead	H	498.572	100	1.026
D5	FAM	LH Bead	I	498.572	100	0.967
D6	FAM	LH Bead	J	498.572	27.91	2.043
D7	FAM	LH Bead	K	498.572	25.36	3.148
D8	FAM	LH Bead	L	498.572	25.29	3.312
E1	FAM	LH Bead	E	498.572	100	1.201
E2	FAM	LH Bead	F	498.572	27.86	1.972
E3	FAM	LH Bead	G	498.572	32.8	1.468
E4	FAM	LH Bead	H	498.572	100	0.987
E5	FAM	LH Bead	I	498.572	100	1.033
E6	FAM	LH Bead	J	498.572	29.41	1.846
E7	FAM	LH Bead	K	498.572	29.1	2.487
E8	FAM	LH Bead	L	498.572	26.21	2.857
F1	FAM	LH Bead	E	498.572	100	1.024
F2	FAM	LH Bead	F	498.572	29.54	1.872
F3	FAM	LH Bead	G	498.572	33.58	1.475

TABLE 4

Well	Dye	Bead	Capture Probe	Threshold	CT (dR)	S/N
F4	FAM	LH Bead	H	498.572	100	1.025
F5	FAM	LH Bead	I	498.572	100	0.971
F6	FAM	LH Bead	J	498.572	26.25	2.702
F7	FAM	LH Bead	K	498.572	25.92	2.807
F8	FAM	LH Bead	L	498.572	26.44	2.89
A1	TAMRA	RH Bead	R-AA	749.768	25.89	6.842
A2	TAMRA	RH Bead	R-No TCO	749.768	26.2	5.66
A3	TAMRA	LH Bead	L-AA	749.768	25.19	8.137
A4	TAMRA	LH Bead	L-No TCO	749.768	100	0.936
A5	TAMRA	LH Bead	A	749.768	100	0.941
A6	TAMRA	LH Bead	B	749.768	100	1.059
A7	TAMRA	LH Bead	C	749.768	100	1.163
A8	TAMRA	LH Bead	D	749.768	27.01	6.466
B1	TAMRA	RH Bead	R-AA	749.768	67.16	6.942
B2	TAMRA	RH Bead	R-No TCO	749.768	27.23	5.931
B3	TAMRA	LH Bead	L-AA	749.768	25.46	7.903
B4	TAMRA	LH Bead	L-No TCO	749.768	100	0.961
B5	TAMRA	LH Bead	A	749.768	100	1.102
B6	TAMRA	LH Bead	B	749.768	100	1.012
B7	TAMRA	LH Bead	C	749.768	100	1.087
B8	TAMRA	LH Bead	D	749.768	28.34	5.789
C1	TAMRA	RH Bead	R-AA	749.768	26.5	6.553
C2	TAMRA	RH Bead	R-No TCO	749.768	28.19	5.561
C3	TAMRA	LH Bead	L-TCO	749.768	28.36	6.119
C4	TAMRA	LH Bead[1]	L-No TCO	749.768	100	0.957
C5	TAMRA	LH Bead	A	749.768	100	0.956
C6	TAMRA	LH Bead	B	749.768	100	1.001
C7	TAMRA	LH Bead	C	749.768	100	1.038
C8	TAMRA	LH Bead	D	749.768	29.14	5.602
D1	TAMRA	LH Bead	E	749.768	100	0.964
D2	TAMRA	LH Bead	F	749.768	27.73	5.858
D3	TAMRA	LH Bead	G	749.768	39.57	2.742
D4	TAMRA	LH Bead	H	749.768	100	0.956
D5	TAMRA	LH Bead	I	749.768	100	0.949

TABLE 4

Well	Dye	Bead	Capture Probe	Threshold	CT (dR)	S/N
D6	TAMRA	LH Bead	J	749.768	38.02	2.421
D7	TAMRA	LH Bead	K	749.768	27.09	6.539
D8	TAMRA	LH Bead	L	749.768	29.93	5.103
E1	TAMRA	LH Bead	E	749.768	100	0.989
E2	TAMRA	LH Bead	F	749.768	30.19	4.704
E3	TAMRA	LH Bead	G	749.768	73	2.309
E4	TAMRA	LH Bead	H	749.768	100	0.955
E5	TAMRA	LH Bead	I	749.768	100	0.951
E6	TAMRA	LH Bead	J	749.768	100	2.042
E7	TAMRA	LH Bead	K	749.768	30.84	5.249
E8	TAMRA	LH Bead	L	749.768	31.28	4.523
F1	TAMRA	LH Bead	E	749.768	100	0.971
F2	TAMRA	LH Bead	F	749.768	29.49	4.952
F3	TAMRA	LH Bead	G	749.768	40.29	2.189
F4	TAMRA	LH Bead	H	749.768	100	0.957
F5	TAMRA	LH Bead	I	749.768	100	0.948
F6	TAMRA	LH Bead	J	749.768	42.28	2.058
F7	TAMRA	LH Bead	K	749.768	28.85	5.364
F8	TAMRA	LH Bead	L	749.768	63.23	4.611

[1]RH bead = bead + D-nucleic acid
[2]LH bead = bead + L-nucleic acid
Threshold: number above which sample is called reactive
S/N: signal/noise

<u>L-TCOs #M – V</u>

[0084] Target capture reactions were performed substantially as described above for L_TCOs A-L. Based on FAM Ct and FAM Signal/ Noise (TABLE 5), all target nucleic acids captured with L-TCOs M-V amplified well. For the No-TCO control, the D-ribose bead showed non-specific binding of HIV-1 and internal control, while the L-ribose bead did not. This observation indicates that nucleic acids having sequence portions that are substantially complementary to the D-nucleic acid immobilized probe, will hybridize thereto. Captured nucleic acids, then, comprise target nucleic acids and nucleic acids that hybridized directly to the D-nucleic acid immobilized probe.

[0085] Summary: All L-TCOs, except for L-TCO I yielded a Ct time, indicating target binding, and were selected for use in the L-ribose based TCR. In contrast to the D-immobilized probes, the L-immobilized probes do not directly bind to target nucleic acids, reducing the possibility of inadvertently capturing an analyzing non-target sequences.

TABLE 5

Well	Dye	Bead	TCO	Rlast/Rfirst	Threshold (dR)	Ct (dR)
A1	FAM	RH Bead	AA	7.943	347.946	28.83
A2	FAM	RH Bead	No TCO	7.499	347.946	30.64
A3	FAM	LH Bead	AA	7.853	347.946	28.35
A4	FAM	LH Bead	No TCO	0.997	347.946	.
A5	FAM	LH Bead	A	3.231	347.946	34.04
A6	FAM	LH Bead	B	0.953	347.946	.
A7	FAM	LH Bead	C	3.694	347.946	32.97
A8	FAM	LH Bead	D	3.63	347.946	32.99
B1	FAM	RH Bead	AA	7.998	347.946	27.93
B2	FAM	RH Bead	No TCO	6.255	347.946	30.9
B3	FAM	LH Bead	NA0138	7.171	347.946	29.39
B4	FAM	LH Bead	No TCO	0.947	347.946	.
B5	FAM	LH Bead	A	3.016	347.946	34.58
B6	FAM	LH Bead	B	1.464	347.946	40
B7	FAM	LH Bead	C	2.989	347.946	31.94
B8	FAM	LH Bead	D	2.514	347.946	35.99
C1	FAM	RH Bead	AA	7.939	347.946	28.86
C2	FAM	RH Bead	No TCO	7.19	347.946	29.88
C3	FAM	LH Bead	AA	7.389	347.946	29.26
C4	FAM	LH Bead	No TCO	1.898	347.946	37.62
C5	FAM	LH Bead	A	1.981	347.946	39.21
C6	FAM	LH Bead	B	1.761	347.946	38.7

TABLE 5

Well	Dye	Bead	TCO	Rlast/Rfirst	Threshold (dR)	Ct (dR)
C7	FAM	LH Bead	C	3.894	347.946	33.69
C8	FAM	LH Bead	D	3.43	347.946	35.05
D1	FAM	LH Bead	E	1.986	347.946	37.56
D2	FAM	LH Bead	F	6.597	347.946	31.16
D3	FAM	LH Bead	G	5.361	347.946	31.84
D4	FAM	LH Bead	H	2.537	347.946	35.8
D5	FAM	LH Bead	I	0.965	347.946	.
D6	FAM	LH Bead	J	4.871	347.946	31.92
D7	FAM	LH Bead	K	7.644	347.946	29.66
D8	FAM	LH Bead	L	6.571	347.946	29.78
E1	FAM	LH Bead	E	2.977	347.946	34.94
E2	FAM	LH Bead	F	5.858	347.946	31.01
E3	FAM	LH Bead	G	3.673	347.946	33.93
E4	FAM	LH Bead	H	1.886	347.946	37.86
E5	FAM	LH Bead	I	0.967	347.946	.
E6	FAM	LH Bead	J	6.228	347.946	31.66
E7	FAM	LH Bead	K	7.32	347.946	29.86
E8	FAM	LH Bead	L	6.758	347.946	29.51
F1	FAM	LH Bead	E	1.677	347.946	38.92
F2	FAM	LH Bead	F	6.231	347.946	31.44
F3	FAM	LH Bead	G	4.005	347.946	33.79
F4	FAM	LH Bead	H	0.99	347.946	.
F5	FAM	LH Bead	I	0.953	347.946	.
F6	FAM	LH Bead	J	5.917	347.946	31.76
F7	FAM	LH Bead	K	7.373	347.946	30.29
F8	FAM	LH Bead	L	6.87	347.946	31.02
A1	TAMRA	RH Bead	AA	9.192	3056.499	36.11

TABLE 5

Well	Dye	Bead	TCO	Rlast/Rfirst	Threshold (dR)	Ct (dR)
A2	TAMRA	RH Bead	No TCO	7.869	3056.499	41.38
A3	TAMRA	LH Bead	AA	9.389	3056.499	39.46
A4	TAMRA	LH Bead	No TCO	0.918	3056.499	.
A5	TAMRA	LH Bead	A	0.912	3056.499	.
A6	TAMRA	LH Bead	B	1.2	3056.499	.
A7	TAMRA	LH Bead	C	1.18	3056.499	.
A8	TAMRA	LH Bead	D	9.158	3056.499	39.62
B1	TAMRA	RH Bead	AA	9.286	3056.499	38.41
B2	TAMRA	RH Bead	No TCO	7.174	3056.499	42.55
B3	TAMRA	LH Bead	AA	8.932	3056.499	40.79
B4	TAMRA	LH Bead	No TCO	0.907	3056.499	.
B5	TAMRA	LH Bead	A	0.916	3056.499	.
B6	TAMRA	LH Bead	B	1.183	3056.499	.
B7	TAMRA	LH Bead	C	1.165	3056.499	.
B8	TAMRA	LH Bead	D	8.728	3056.499	40.93
C1	TAMRA	RH Bead	AA	8.739	3056.499	39.83
C2	TAMRA	RH Bead	No TCO	7.73	3056.499	41.78
C3	TAMRA	LH Bead	AA	7.776	3056.499	42.15
C4	TAMRA	LH Bead	No TCO	0.92	3056.499	.
C5	TAMRA	LH Bead	A	0.917	3056.499	.
C6	TAMRA	LH Bead	B	1.183	3056.499	.
C7	TAMRA	LH Bead	C	1.157	3056.499	.
C8	TAMRA	LH Bead	D	7.99	3056.499	42.22
D1	TAMRA	LH Bead	E	0.958	3056.499	.
D2	TAMRA	LH Bead	F	7.978	3056.499	42.45
D3	TAMRA	LH Bead	G	3.762	3056.499	55.35
D4	TAMRA	LH Bead	H	0.914	3056.499	.

TABLE 5

Well	Dye	Bead	TCO	Rlast/Rfirst	Threshold (dR)	Ct (dR)
D5	TAMRA	LH Bead	I	0.934	3056.499	.
D6	TAMRA	LH Bead	J	4.376	3056.499	52.04
D7	TAMRA	LH Bead	K	8.671	3056.499	40.79
D8	TAMRA	LH Bead	L	7.202	3056.499	44.53
E1	TAMRA	LH Bead	E	0.963	3056.499	.
E2	TAMRA	LH Bead	F	7.228	3056.499	43.95
E3	TAMRA	LH Bead	G	3.573	3056.499	56.69
E4	TAMRA	LH Bead	H	0.916	3056.499	.
E5	TAMRA	LH Bead	I	0.904	3056.499	.
E6	TAMRA	LH Bead	J	3.771	3056.499	55.28
E7	TAMRA	LH Bead	K	8.813	3056.499	40.73
E8	TAMRA	LH Bead	L	8.317	3056.499	42.39
F1	TAMRA	LH Bead	E	0.958	3056.499	.
F2	TAMRA	LH Bead	F	7.651	3056.499	43.14
F3	TAMRA	LH Bead	G	3.981	3056.499	54.21
F4	TAMRA	LH Bead	H	0.912	3056.499	.
F5	TAMRA	LH Bead	I	0.919	3056.499	.
F6	TAMRA	LH Bead	J	4.112	3056.499	53.54
F7	TAMRA	LH Bead	K	8.104	3056.499	41.9
F8	TAMRA	LH Bead	L	8.13	3056.499	43.69

Internal control was removed from the assay for the remainder of the studies.

Example 6

This example compares the performance of D- and L-capture probes in capturing varying copy number of an HIV target nucleic acid assessed by quantitative PCR of captured target.

a. L-TCR Preparation with 24 L-Ribose TCOs and Evaluation (+/-) Elution

[0086] An L-ribose target capture reagent (L-TCR) was prepared. The L-TCR consisted of about 20 ug/rxn beads and 0.1 pmol of each L-TCO in TABLE 3 except for TCO-I. TCOs AA and BB were also prepared as positive controls having D-nucleic acid second segments and combined with beads attached to D-nucleic acid immobilized probes.

[0087] Target was eluted from beads into 75 uL of water and compared to beads carried into the Amp reaction without an elution step.

[0088] A 2.5 uL quantity of the material eluted into water was spiked into 75 uL of Amp reagent for the RTMA assay. Only 1/30th of the starting eluted material was assayed. Here the minimum tested with elution was 2000 copies/mL or 1000 copies/rxn. The plate setup for the evaluation of L-TCR performance with and without target elution is shown below in Table 6. The numbers within the plate represent the copies/mL of HIV present at that well position.

TABLE 6

	1.82 pmol each of 3 Standard RH-TCOs						0.1 pmol each of 24 LH-TCOs					
	No Elution			+ Elution			No Elution			+ Elution		
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	0	0	0	0	0	0	0	0	0	0
B	30	30	30	1k	1k	1k	1k	1k	1k	1k	1k	1k
C	100	100	100	3k	3k	3k	3k	3k	3k	3k	3k	3k
D	300	300	300	10k	10k	10k	10k	10k	10k	10k	10k	10k
E	1k	1k	1k	30k	30k	30k	30k	30k	30k	30k	30k	30k
F	3k	3k	3k	--	--	--	--	--	--	--	--	--
G	10k	10k	10k	--	--	--	--	--	--	--	--	--
H	--	--	--	--	--	--	--	--	--	--	--	--

[0089] Following target capture, real-time RTMA was performed as generally described above, and the results are present in Tables 7 and 8. Ct Times are shown in Table 7.

[0090] Table 8 shows Ct Emergence times comparing D-TCR (RH) and L-TCR (LH) with and without elution of target at several target concentrations. The starting copy number for the (+) elution data is 1/30th of the total eluted material i.e. copies present at the start of RTMA. Fig. 2 shows the same data shown in Table 8. Fig. 2 shows L-TCR plotted next to D-TCR for comparison of CT emergence times. The data shown in Fig. 2 are broken out into (-) Elution (beads carried into reaction) and (+) Elution (1/30th sample into reaction).

[0091] For “No-Elution”, L-TCR performance based on Ct was similar to D-TCR at 1000 and 3000 c/rxn. At 10,000 c/rxn, D-TCR was slightly faster. For the eluted samples, L-TCR performance based on Ct was similar to D-TCR at 100 and 333 c/rxn. L-TCR was faster at 33 c/rxn and D-TCR was faster at 1000 c/rxn. The L-ribose based TCR successfully detected 2000 copies/mL using only a 2.5 uL volume (50 copies) of HIV-1 inactivated virus.

TABLE 7

Well	Well Name	Dye	Assay	Threshold (dR)	Ct (dR)	Quantity (copies) before amp.
A1	---	FAM	FAM	5331.279	No Ct	No Ct
A2	---	FAM	FAM	5331.279	No Ct	No Ct
A3	---	FAM	FAM	5331.279	No Ct	No Ct
A4	RH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A5	RH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A6	RH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A7	LH - No Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A8	LH - No Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A9	LH - No Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A10	LH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A11	LH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
A12	LH + Elution - 0	FAM	FAM	5331.279	No Ct	No Ct
B1	30	FAM	FAM	5331.279	34.3	1.22E+02
B2	30	FAM	FAM	5331.279	34.58	1.05E+02
B3	30	FAM	FAM	5331.279	35.88	5.17E+01
B4	RH + Elution - 1k	FAM	FAM	5331.279	33.36	2.04E+02
B5	RH + Elution - 1k	FAM	FAM	5331.279	32.95	2.54E+02
B6	RH +	FAM	FAM	5331.279	37	2.82E+01

TABLE 7

Well	Well Name	Dye	Assay	Threshold (dR)	Ct (dR)	Quantity (copies) before amp.
	Elution - 1k					
B7	LH - No Elution - 1k	FAM	FAM	5331.279	29.34	1.80E+03
B8	LH - No Elution - 1k	FAM	FAM	5331.279	29.71	1.48E+03
B9	LH - No Elution - 1k	FAM	FAM	5331.279	29.77	1.43E+03
B10	LH + Elution - 1k	FAM	FAM	5331.279	31.92	4.46E+02
B11	LH + Elution - 1k	FAM	FAM	5331.279	32.34	3.54E+02
B12	LH + Elution - 1k	FAM	FAM	5331.279	29.97	1.29E+03
C1	---	FAM	FAM	5331.279	33.88	1.00E+02
C2	---	FAM	FAM	5331.279	35.48	1.00E+02
C3	---	FAM	FAM	5331.279	35.7	1.00E+02
C4	RH + Elution - 3k	FAM	FAM	5331.279	30.87	7.87E+02
C5	RH + Elution - 3k	FAM	FAM	5331.279	31.07	7.04E+02
C6	RH + Elution - 3k	FAM	FAM	5331.279	30.35	1.04E+03
C7	LH - No Elution - 3k	FAM	FAM	5331.279	30.37	1.03E+03
C8	LH - No Elution - 3k	FAM	FAM	5331.279	29.86	1.36E+03
C9	LH - No Elution - 3k	FAM	FAM	5331.279	28.67	2.60E+03
C10	LH + Elution - 3k	FAM	FAM	5331.279	31.74	4.91E+02
C11	LH + Elution - 3k	FAM	FAM	5331.279	32.12	3.98E+02
C12	LH + Elution - 3k	FAM	FAM	5331.279	30.5	9.62E+02
D1	300	FAM	FAM	5331.279	31.21	6.54E+02
D2	300	FAM	FAM	5331.279	31.04	7.16E+02
D3	300	FAM	FAM	5331.279	32.08	4.09E+02
D4	RH + Elution - 10k	FAM	FAM	5331.279	30.32	1.06E+03

TABLE 7

Well	Well Name	Dye	Assay	Threshold (dR)	Ct (dR)	Quantity (copies) before amp.
D5	RH + Elution - 10k	FAM	FAM	5331.279	30.07	1.21E+03
D6	RH + Elution - 10k	FAM	FAM	5331.279	29.44	1.71E+03
D7	LH - No Elution - 10k	FAM	FAM	5331.279	26.94	6.64E+03
D8	LH - No Elution - 10k	FAM	FAM	5331.279	26.87	6.88E+03
D9	LH - No Elution - 10k	FAM	FAM	5331.279	27.4	5.18E+03
D10	LH + Elution - 10k	FAM	FAM	5331.279	30.18	1.14E+03
D11	LH + Elution - 10k	FAM	FAM	5331.279	29.72	1.47E+03
D12	LH + Elution - 10k	FAM	FAM	5331.279	29.1	2.06E+03
E1	---	FAM	FAM	5331.279	29.22	1.00E+03
E2	---	FAM	FAM	5331.279	29.67	1.00E+03
E3	---	FAM	FAM	5331.279	30.27	1.00E+03
E4	RH + Elution - 30k	FAM	FAM	5331.279	28.06	3.61E+03
E5	RH + Elution - 30k	FAM	FAM	5331.279	27.76	4.26E+03
E6	RH + Elution - 30k	FAM	FAM	5331.279	28.24	3.28E+03
E7	LH - No Elution - 30k	FAM	FAM	5331.279	26.36	9.12E+03
E8	LH - No	FAM	FAM	5331.279	25.88	1.18E+04

TABLE 7

Well	**Well Name**	**Dye**	**Assay**	**Threshold (dR)**	**Ct (dR)**	**Quantity (copies) before amp.**
	Elution - 30k					
E9	LH - No Elution - 30k	FAM	FAM	5331.279	26.24	9.68E+03
E10	LH + Elution - 30k	FAM	FAM	5331.279	29.16	1.99E+03
E11	LH + Elution - 30k	FAM	FAM	5331.279	28.42	2.97E+03
E12	LH + Elution - 30k	FAM	FAM	5331.279	28.4	3.01E+03
F1	3000	FAM	FAM	5331.279	28.72	2.53E+03
F2	3000	FAM	FAM	5331.279	28.83	2.39E+03
F3	3000	FAM	FAM	5331.279	29.18	1.97E+03
F4	---	FAM	FAM	5331.279	No Ct	No Ct
F5	---	FAM	FAM	5331.279	No Ct	No Ct
F6	---	FAM	FAM	5331.279	No Ct	No Ct
F7	---	FAM	FAM	5331.279	No Ct	No Ct
F8	---	FAM	FAM	5331.279	No Ct	No Ct
F9	---	FAM	FAM	5331.279	No Ct	No Ct
F10	---	FAM	FAM	5331.279	No Ct	No Ct
F11	---	FAM	FAM	5331.279	No Ct	No Ct
F12	---	FAM	FAM	5331.279	No Ct	No Ct
G1	---	FAM	FAM	5331.279	26.66	1.00E+04
G2	---	FAM	FAM	5331.279	26.46	1.00E+04
G3	---	FAM	FAM	5331.279	26.49	1.00E+04
G4	---	FAM	FAM	5331.279	No Ct	No Ct
G5	---	FAM	FAM	5331.279	No Ct	No Ct
G6	---	FAM	FAM	5331.279	No Ct	No Ct
G7	---	FAM	FAM	5331.279	No Ct	No Ct
G8	---	FAM	FAM	5331.279	No Ct	No Ct
G9	---	FAM	FAM	5331.279	No Ct	No Ct
G10	---	FAM	FAM	5331.279	No Ct	No Ct
G11	---	FAM	FAM	5331.279	No Ct	No Ct
G12	---	FAM	FAM	5331.279	No Ct	No Ct
H1	---	FAM	FAM	5331.279	No Ct	No Ct

TABLE 7

Well	**Well Name**	**Dye**	**Assay**	**Threshold (dR)**	**Ct (dR)**	**Quantity (copies) before amp.**
H2	---	FAM	FAM	5331.279	No Ct	No Ct
H3	---	FAM	FAM	5331.279	No Ct	No Ct
H4	---	FAM	FAM	5331.279	No Ct	No Ct
H5	---	FAM	FAM	5331.279	No Ct	No Ct
H6	---	FAM	FAM	5331.279	No Ct	No Ct
H7	---	FAM	FAM	5331.279	No Ct	No Ct
H8	---	FAM	FAM	5331.279	No Ct	No Ct
H9	---	FAM	FAM	5331.279	No Ct	No Ct
H10	---	FAM	FAM	5331.279	No Ct	No Ct
H11	---	FAM	FAM	5331.279	No Ct	No Ct
H12	---	FAM	FAM	5331.279	No Ct	No Ct

TABLE 8				
	Control Condition: 1.82 pmol each of 3 Standard RH-TCOs/20 ug beads/rxn		Test Condition: 0.1 pmol each of 24 LH-TCOs/20 ug beads/rxn	
	Ct Emergence time		Ct Emergence time	
Sample Copies/ 500 uL	No Elution	+ Elution	No Elution	+ Elution
0	No Ct	No Ct	No Ct	No Ct
0	No Ct	No Ct	No Ct	No Ct
0	No Ct	No Ct	No Ct	No Ct
1000	26.72	30.77	26.79	29.41
1000	27.09	30.53	27.12	29.9
1000	27.66	32.21	27.21	27.57
3000	26.11	28.2	27.6	29.24
3000	26.17	28.46	27.07	29.77
3000	26.63	27.85	26.11	28.18
10000	24.17	27.87	24.45	27.64
10000	23.94	27.47	24.43	27.14
10000	23.99	26.84	24.81	26.54
30000		25.47	23.74	26.63
30000		25.19	23.25	25.86
30000		25.75	23.72	25.81
30	31.29			
30	31.66			
30	32.55			
100	31.11			
100	32.42			
100	32.62			
300	28.55			
300	28.41			
300	29.41			

Example 7

[0092] A plasma sample from a human infected with HIV is subject to parallel processing and sequence analysis to compare L- and D- capture probes and complementary L- and D- immobilized probes. The immobilized probes include homopolymers of thymine L-deoxyribose and thymine D-deoxyribose (L- and D- polyT) respectively. The capture probes include homopolymers of adenine L-deoxyribose and adenine D-deoxyribose (L- and D- polyA) respectively. Plasma is treated with detergent to solubilize the viral envelope, denature proteins and release viral genomic RNA. Both capture probes including a target-binding segment specific for a conserved region of an HIV genomic RNA target. Parallel samples are contacted with capture and immobilized probes under the conditions as described in Example 5 or as generally described in this application. In both samples being processed, target HIV genomic RNA binds to the capture probe which binds to the immobilized probe. However, in the sample treated with the D-capture probe and D-immobilized probe, poly-A mRNA in the sample unrelated to the HIV genomic RNA target, binds directly to the immobilized probe by D-polyA binding to D-polyT. No such binding occurs in the sample receiving L-capture probe and L-immobilized probe.

[0093] The captured nucleic acids are subjected to amplification (e.g., RT-PCR or RTMA) using primers specific for the intended HIV genomic RNA target. The amplification enriches for the intended target relative to unrelated contaminant nucleic acids that directly bound the D-immobilized probe. However, significant amounts of contaminant nucleic acids are still present after the amplification from the nucleic acids captured by the D-immobilized probe but not from the amplification product captured by the L-immobilized probe.

[0094] The nucleic acids resulting from amplification are then ligated to SMRT-bell™ adapters and subjected to single-molecule real-time sequencing (Korlach et al., *Nucleosides, Nucleotides and Nucleic Acids*, 27:1072–1083 (2008), US 7,181,122, 7,302,146, and 7,313,308). In such a format, circular templates are sequenced individually and an incorporated nucleobase unit is detected in real time before incorporation of the next incorporated nucleobase unit. Sequencing of an individual templates can take place in a cylindrical metallic chamber known as a zero mode waive guide, and many such individual templates each in its own zero mode waive guide can be sequenced in parallel.

[0095] The sequences identified from the sample treated with L-nucleotide probes are substantially all the intended HIV target nucleic acid. Individual sequencing reads may differ from one another due to the sample containing a mixed population of sequence variants and because of sequencing errors. Individual sequence reads are compiled to determine the sequences of individual viral variants present in the population including a majority species and minority species represented at lower frequency. The existence of minority species may provide an indication of emerging drug resistances. The sequences identified from the sample treated with D-nucleotide probes also include such sequences of the intended target subject to similar variation due to viral variants and sequencing errors. However, sequences from non-target polyA mRNA are also present in the sample. The presence of such spurious sequences complicates analysis, for example, in distinguishing genuine variation between HIV sequences and sequencing errors.

[0096] This description contains a sequence listing in electronic form in ASCII text format. A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

What is claimed is:

1. A method of capturing a target nucleic acid, comprising:

contacting a target nucleic acid with a capture probe and an immobilized probe, the capture probe comprising a first segment that binds to the target nucleic acid and a second segment that binds to the immobilized probe, wherein the second segment of the capture probe and the immobilized probe comprise poly-A and poly-T L-nucleic acids that can hybridize to one another, wherein the first segment of the capture probe binds to the target nucleic acid, and the second segment of the capture probe binds to the immobilized probe with a lower melting temperature than the first segment of the capture probe binds to the target nucleic acid, thereby capturing the target nucleic acid.

2. The method of claim 1, wherein the first segment includes a D- nucleic acid of at least 10 D-nucleobase units complementary to the target nucleic acid.

3. The method of claim 2, wherein the D-nucleic acid comprises any of adenine-D-deoxyribose, guanine D-deoxyribose, thymine D-deoxyribose and cytosine D-deoxyribose.

4. The method of claim 1, wherein the first segment includes a D-nucleic acid of 10-30 D-nucleobase units complementary to the target nucleic acid.

5. The method of any one of claims 1 to 4, wherein the first segment binds non-specifically to the target nucleic acid.

6. The method of claim 5, wherein the first segment includes a random sequence of D-nucleobase units that binds nonspecifically to the target nucleic acid.

7. The method of any one of claims 1 to 6, wherein the second segment includes an L-nucleic acid of at least six L-nucleobase units complementary to an L-nucleic acid of at least six L-nucleobase units in the immobilized probe.

8. The method of any one of claims 1 to 6, wherein the second segment includes an L-nucleic acid of 10-30 L-nucleobase units complementary to an L-nucleic acid of 10-30 contiguous L-nucleobase units in the immobilized probe.

9. The method of claim 8, wherein the second segment comprises a homopolymer of 10-30 L-adenine nucleobase units and the immobilized probe comprises a homopolymer of 10-30 L-thymine nucleobase units.

10. The method of any one of claims 1 to 9, wherein the target nucleic acid is contacted with the capture probe and immobilized probe simultaneously.

11. The method of any one of claims 1 to 9, wherein the target nucleic acid is contacted with the capture probe before the immobilized probe.

12. The method of any one of claims 1 to 11, wherein the binding of the target nucleic acid to the capture probe occurs under first hybridization conditions and the binding of the capture probe to the immobilized probe occurs under second hybridization conditions.

13. The method of claim 12, wherein the first conditions are more stringent than the second conditions.

14. The method of any one of claims 1 to 9, wherein the binding of the target nucleic acid to the capture probe and the binding of the capture probe to the immobilized probe occur under the same hybridization conditions.

15. The method of any one of claims 1 to 14, wherein the immobilized probe is immobilized to a magnetic bead.

16. The method of any one of claims 1 to 15, wherein the target nucleic acid is provided as a component of a sample and the method further comprising separating the captured target nucleic acid from other components of the sample.

17. The method of claim 16, further comprising dissociating the captured target nucleic acid from the immobilized probe.

18. The method of claim 17, further comprising amplifying the target nucleic acid.

19. The method of claim 18, wherein the amplifying is performed after dissociating the target nucleic acid from the capture probe.

20. The method of claim 17, further comprising contacting the target nucleic acid with a detection probe and detecting the detection probe.

21. The method of any one of claims 17 to 20, wherein the target nucleic is contacted with a detection probe after dissociating the target nucleic acid from the capture probe.

22. The method of any one of claims 1 to 21, wherein the target nucleic acid is an HIV nucleic acid.

23. The method of any one of claims 1 to 22, wherein the sample includes mRNA or a nucleic acid derived therefrom.

24. A hybridization complex comprising:

(i) a capture probe comprising a first segment of at least 10 D-nucleobase units that can hybridize to a target nucleic acid and a second segment comprising a homopolymer of at least 10 L-nucleobase units

(ii) immobilized probe comprising a homopolymer of at least 10 L-nucleobase units linked to a support

wherein the capture probe and immobilized probe comprise complementary homopolyeric L-nucleic acids that can hybridize to one another, with a lower melting temperature than the first segment of the capture probe binds to the target nucleic acid, wherein the homopolymeric L-nucleic acids are polyA and polyT.

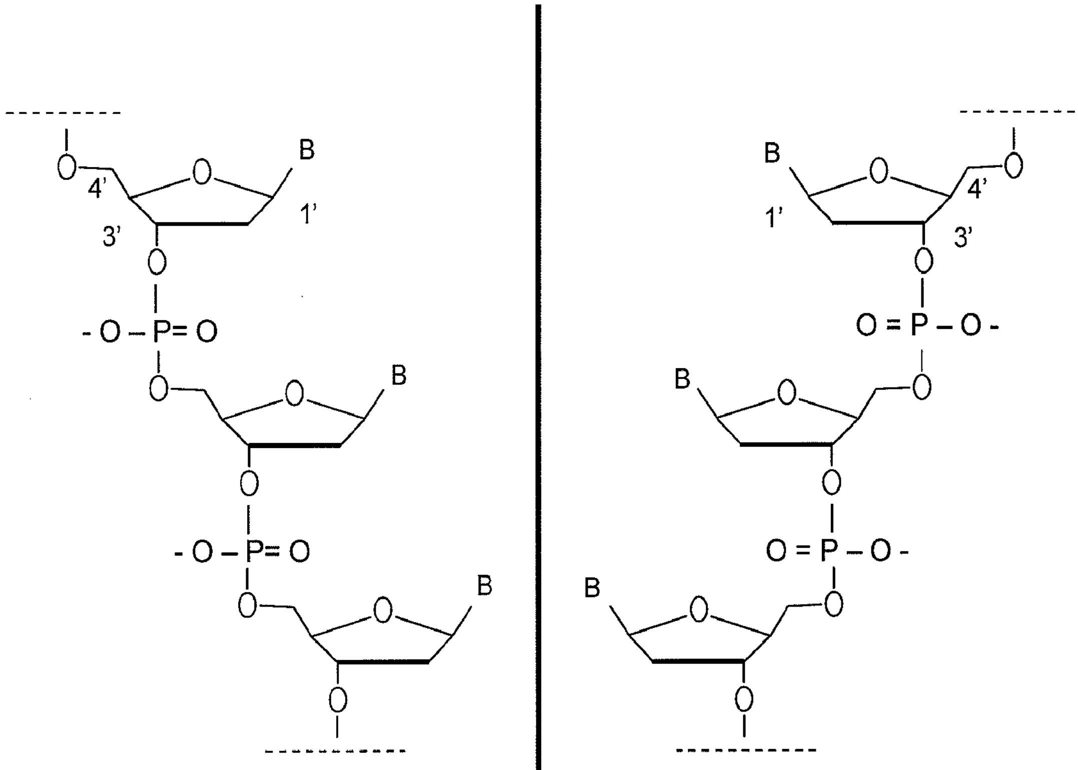
25. The hybridization complex of claim 24, wherein the support is a magnetic particle.

26. The hybridization complex of claim 24 or 25, wherein the immobilized probe comprises a homopolymer of thymine nucleobase units.

27. A kit comprising:

a capture probe comprising a first segment including a D-nucleic acid that hybridizes to a target nucleic acid and a second segment including a homopolymeric L-nucleic acid and an immobilized probe comprising an L-nucleic acid comprising a complementary homopolymeric L-nucleic acid,, wherein the homopolymeric L-nucleic acids are poly-A and poly-T and can hybridize to one another via hybridization of the complementary homopolymeric L-nucleic acids with a lower melting temperature than the first segment of the capture probe hybridizes to the target nucleic acid.

28. The kit of claim 27, wherein the homopolymeric L-nucleic acid of the capture probe is a homopolymer of adenine nucleobase units and the complementary homopolymeric L-nucleic acid of the immobilized probe is a homopolymer of thymine nucleobase units.



D-form DNA

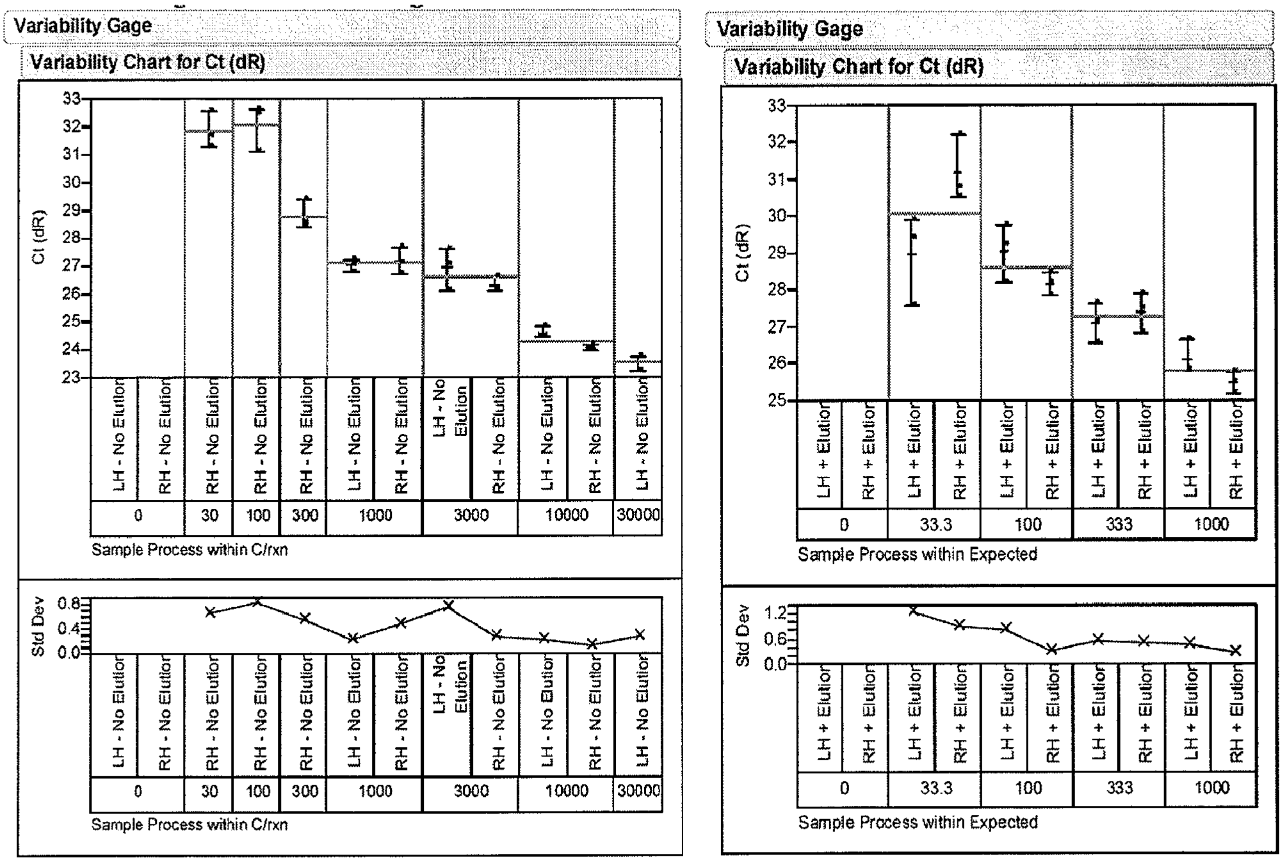
1'R, 3'S, 4'R

L-form DNA

1'S, 3'R, 4'S

Figure 1

Fig. 2


Variability Gage
Variability Chart for Ct (dR)
Ct (dR)
Std Dev
LH - No Elution
RH - No Elution
0
30
100
300
1000
3000
10000
30000
Sample Process within C/rxn
Variability Gage
Variability Chart for Ct (dR)
Ct (dR)
Std Dev
LH + Elution
RH + Elution
0
33.3
100
333
1000
Sample Process within Expected