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(54) Title: DETECTION OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1			
(57) Abstract Amplification oligonucleotides and hybridization assay probes which distinguish Human Immunodeficiency Virus type 1 from other viruses.			

DESCRIPTIONDetection of Human Immunodeficiency Virus Type 1

This application is a continuation in part of Kacian and Fultz, entitled "Nucleic Acid Sequence Amplification Methods," U.S. Serial No. 07/379,501, filed 7/11/89, and Kacian and Fultz, entitled "Nucleic Acid Sequence
5 Amplification Methods," U.S. Serial No. 07/550,837, filed 7/10/90, both hereby incorporated by reference herein.

Field of the Invention

This invention relates to the design and construction of amplification oligonucleotides and probes to Human
10 Immunodeficiency Virus Type 1 (HIV), which allow detection of the organism in a test sample.

Background of the Invention

This section provides a brief outline of relevant areas. None of the art cited or referred to is admitted
15 to be prior art to the claims. Laboratory diagnosis of Human Immunodeficiency Virus Type 1 in humans is currently performed by demonstration of the presence of viral antigen (p24) or anti-HIV-1 antibodies in serum. Direct detection of viral DNA, however, is a more useful
20 diagnostic tool in some populations, such as infants born to seropositive mothers. Detection of viral DNA is more rapid and less hazardous than culture. Direct hybridization lacks adequate sensitivity in most patients (Shaw et al. Science 226:1165-1171, 1984). Many references mention
25 oligonucleotides said to have use in detection of Human Immunodeficiency Virus. Most of these references also mention the use of polymerase chain reaction (PCR). These references include the following: Kwok et al., J. Virol. 61: 1690-1694, 1987; Agius et al., J. Virol. Meth.,
30:141-150, 1990; Albert and Fenyo, J. Clin. Microbiol. 28:1560-1564, 1990; Bell and Ratner, AIDS Res. and Human



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- 5 Biochem. Biophys. Res. Comm. 167:244-250, 1990; Ferrer-Le-Coeur et al., Thrombosis and Haemostasis 65:478-482, 1991; Goswami et al., AIDS 5:797-803, 1991; Grankvist et al., AIDS 5:575-578, 1991; Guatelli et al., J. Virol. 64:4093-4098, 1990; Hart et al., Lancet 2 (8611):596-599, 1988;
- 10 Holland et al., Proc. Natl. Acad. Sci. USA, 88:7276-7280, 1991; Keller et al., Anal. Biochem. 177:27-32, 1989; Kumar et al., AIDS Res. and Human Retroviruses 5:345-354, 1989; Linz et al., J. Clin. Chem. Clin. Biochem. 28:5-13, 1990; Mano and Chermann, Res. Virol. 142:95-104, 1991; Mariotti
- 15 et al., AIDS 4:633-637, 1990; Mariotti et al., Transfusion 30:704-706, 1990; Meyerhans et al., Cell 58:901-910, 1989; Mousset et al., AIDS 4:1225-1230, 1990; Ou et al., Science 239:295-297, 1988; Pang et al., Nature 343:85-89, 1990; Paterlini et al., J. Med. Virol. 30:53-57, 1990; Perrin et
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- 25 64:3792-3803, 1990; Truckenmiller et al., Res. Immunol. 140:527-544, 1989; Van de Perre, et al., New Eng. J. Med. 325:593-598, 1991; Varas et al., BioTechniques 11:384-391, 1991; Velpandi et al., J. Virol. 65:4847-4852, 1991; Williams et al., AIDS 4:393-398, 1990; Zachar et al., J.
- 30 Virol. Meth. 33:391-395, 1991; Zack et al. Cell 61:213-222, 1990; Findlay et al., entitled "Nucleic acid test article and its use to detect a predetermined nucleic acid," PCT/US90/00452; Gingeras et al., entitled "Nucleic acid probe assay methods and compositions," PCT/US87/
- 35 01966; Brakel and Spadoro, entitled "Amplification capture assay," EPO application number 90124738.7, publication number 0 435 150 A2; Moncany and Montagnier, entitled

"Séquences nucléotidiques issues du génome des rétrovirus du typ hiv-1, hiv-2 et siv, et leurs applications notamment pour l'amplification des génomes de ces rétrovirus et pour le diagnostic in-vitro des infections dues à ces virus," EPO application number 90401520.3, publication number 0 403 333 A2; Urdea, entitled "DNA-dependent RNA polymerase transcripts as reporter molecules for signal amplification in nucleic acid hybridization assays," PCT/US91/00213; Musso et al., entitled "Lanthanide chelate-tagged nucleic acid probes," PCT/US88/03735; Chang, entitled "Cloning and expression of HTLV-III DNA," EPO application number 85307260.1, publication number 0 185 444 A2; and Levenson, entitled "Diagnostic kit and method using a solid phase capture means for detecting nucleic acids," EPO application number 89311862.0, publication number 0 370 694; and Sninsky et al., U.S. Patent No. 5,008,182.

Summary of the Invention

This invention discloses novel amplification oligonucleotides and detection probes for the detection of Human Immunodeficiency Virus Type 1. The probes are capable of distinguishing between the Human Immunodeficiency Virus type 1 and its known closest phylogenetic neighbors. The amplification oligonucleotides and probes may be used in an assay for the detection and/or quantitation of Human Immunodeficiency Virus nucleic acid.

It is known that a nucleic acid sequence able to hybridize to a nucleic acid sequence sought to be detected ("target sequence") can serve as a probe for the target sequence. The probe may be labelled with a detectable moiety such as a radioisotope, antigen or chemiluminescent moiety to facilitate detection of the target sequence. A background description of the use of nucleic acid hybridization as a procedure for the detection of particular nucleic acid sequences is provided in Kohne, U.S. Patent No. 4,851,330, and Hogan et al., EPO Patent Application

No. PCT/US87/03009, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral Organisms."

It is also known that hybridization may occur between complementary nucleic acid strands including; DNA/DNA, DNA/RNA, and RNA/RNA. Two single strands of deoxyribo- ("DNA") or ribo- ("RNA") nucleic acid, formed from nucleotides (including the bases adenine (A), cytosine (C), thymidine (T), guanine (G), uracil (U), or inosine (I)), may hybridize to form a double-stranded structure in which the two strands are held together by hydrogen bonds between pairs of complementary bases. Generally, A is hydrogen bonded to T or U, while G is hydrogen bonded to C. At any point along the hybridized strands, therefore, one may find the classical base pairs AT or AU, TA or UA, GC, or CG. Thus, when a first single strand of nucleic acid contains sufficient contiguous complementary bases to a second, and those two strands are brought together under conditions which will promote their hybridization, double-stranded nucleic acid will result. Under appropriate conditions, DNA/DNA, RNA/DNA, or RNA/RNA hybrids may be formed. The present invention includes the use of probes or primers containing nucleotides differing in the sugar moiety, or otherwise chemically modified, which are able to hydrogen bond along the lines described above.

Thus, in a first aspect, the invention features hybridization assay probes able to distinguish Human Immunodeficiency Virus type 1 from other viruses found in human blood or tissues, and amplification oligonucleotides able to selectively amplify Human Immunodeficiency Virus nucleic acid. Specifically, the probes are nucleotide polymers which hybridize to the nucleic acid region of Human Immunodeficiency Virus type 1 corresponding to bases 763-793 of HIV type 1, (HXB2 isolate GenBank accession number K03455), or any of the regions corresponding to bases 1271-1301, 1358-1387, 1464-1489, 1501-1540, 1813-1845, 2969-2999, 3125-3161, 4148-4170, 4804-4832, 5950-5978, 9496-9523, 510-542, and 624-651; preferably, the

oligonucleotide comprises, consists essentially of, or consists of the sequence (reading 5' to 3')

(SEQ ID NO: 1) GACTAGCGGAGGCTAGAAGGAGAGAGATGGG

(SEQ ID NO: 2) GAAGGCTTTCAGCCCAGAAGTAATACCCATG

5 (SEQ ID NO: 3) ATTTGCATGGCTGCTTGATGTCCCCCACT

(SEQ ID NO: 4) CTTCCCCTTGGTTCTCTCATCTGGCC

(SEQ ID NO: 5) GTCATCCATCCTATTTGTTCTGAAGGGTACTAGTAG

(SEQ ID NO: 6) CTCCCTGACATGCTGTCATCATTTCTTCTAGTG

(SEQ ID NO: 7) GTGGAAGCACATTGTACTGATATCTAATCCC

10 (SEQ ID NO: 8) GCTCCTCTATTTTTGTTCTATGCTGCCCTATTTCTAA

(SEQ ID NO: 9) CCTTTGTGTGCTGGTACCCATGC

(SEQ ID NO: 10) CTACTATTCTTTCCCTGCACTGTACCCC

(SEQ ID NO: 11) AAAGCCTTAGGCATCTCCTATGGCAGGAA

(SEQ ID NO: 12) GCAGCTGCTTATATGCAGGATCTGAGGG

15 (SEQ ID NO: 13) CAAGGCAAGCTTTATTGAGGCTTAAGCAGTGGG

(SEQ ID NO: 14) ATCTCTAGCAGTGGCGCCCGAACAGGGA

or RNA equivalents thereto (SEQ. ID. Nos. 67-80), or oligonucleotides complementary thereto (SEQ. ID. Nos. 53-66), or RNA equivalents to the oligonucleotides complementary thereto (SEQ. ID Nos. 81-94).

20 According to a first embodiment of the invention there is provided a detection probe able to detect the presence of Human Immunodeficiency Virus nucleic acid comprising a sequence able to hybridise to an HIV-1 nucleic acid target region selected from the group consisting of:

(SEQ ID NO: 3) ATTTGCATGGCTGCTTGATGTCCCCCACT,

25 (SEQ ID NO: 5) GTCATCCATCCTATTTGTTCTGAAGGGTACTAGTAG,

(SEQ ID NO: 6) CTCCCTGACATGCTGTCATCATTTCTTCTAGTG,

(SEQ ID NO: 7) GTGGAAGCACATTGTACTGATATCTAATCCC,

(SEQ ID NO: 8) GCTCCTCTATTTTTGTTCTATGCTGCCCTATTTCTAA,

(SEQ ID NO: 9) CCTTTGTGTGCTGGTACCCATGC,

30 (SEQ ID NO: 11) AAAGCCTTAGGCATCTCCTATGGCAGGAA,

(SEQ ID NO: 13) CAAGGCAAGCTTTATTGAGGCTTAAGCAGTGGG,

(SEQ ID NO: 14) ATCTCTAGCAGTGGCGCCCGAACAGGGA,

(SEQ ID NO: 83) AGUGGGGGGACAUCAAGCAGCCAUGCAAAU,

(SEQ ID NO: 85) CUACUAGUACCCUUCAGGAACAAAUAGGAUGGAUGAC,

35 (SEQ ID NO: 86) CACUAGAAGAAAUGAUGACAGCAUGUCAGGGAG,

(SEQ ID NO: 87) GGGAUUAGAUUAUCAGUACAAUGUGCUUCCAC,

(SEQ ID NO: 88) UUAGAAAUAGGGCAGCAUAGAACAAAAUAGAGGAGC,

(SEQ ID NO: 89) GCAUGGGUACCAGCACAAAGG,

(SEQ ID NO: 91) UCCUGCCAUAGGAGAUGCCUAAGGCUUU,



(SEQ ID NO: 93) CCCACUGCUUAAGCCUCAAUAAAGCUUGCCUUG, and
 (SEQ ID NO: 94) UCCCUGUUCGGGCGCCACUGCUAGAGAU,

wherein under hybridization assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridise to non-
 5 target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human hepatitis virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-target nucleic acid under said conditions.

The oligonucleotides are used with or without a helper probe as described below.
 10 The use of helper probes (e.g., SEQ. ID. Nos. 15-18) and complementary oligonucleotides to the helper probes (e.g., SEQ. ID. Nos. 95-98) and RNA equivalents thereto (e.g., SEQ. ID. Nos. 132-140) enhances nucleic acid hybridization.

By "consists essentially of" is meant that the probe is provided as a purified nucleic acid which under stringent hybridizing conditions hybridizes with the target
 15 sequence and not with other related target sequences present in either other virus nucleic acids or human nucleic acids. Such a probe may be linked to other nucleic acids which do not affect such hybridization. Generally, it is preferred that the probe is between 15 to 100 (most preferably between 20 and 50) bases in size. It may, however, be provided in a vector.

20 According to a further embodiment of the invention, there is provided a method for determining whether HIV-1 may be present in a sample and distinguishing the presence of HIV-1 from HIV-2 comprising the steps of:

a) contacting said sample with the detection probe in accordance with the first embodiment of the invention; and

25 b) detecting whether said probe:target duplex has formed using said hybridisation conditions to indicate whether HIV-1 may be present in said sample and to distinguish the presence of HIV-1 from HIV-2.



In a related aspect, the invention features the formation of nucleic acid hybrids formed by the hybridization of the probes of this invention with target nucleic acid under stringent hybridization conditions. Stringent hybridization conditions involve the use 0.05 M lithium succinate buffer containing 0.6 M LiCl at 60°C. The hybrids are useful because they allow the specific detection of viral nucleic acid.

In another related aspect, the invention features amplification oligonucleotides useful for specific detection of Human Immunodeficiency Virus type 1 in an amplification assay. The amplification oligonucleotides are complementary to conserved regions of HIV genomic nucleic acid and are nucleotide polymers able to hybridize to regions of the nucleic acid of HIV corresponding to HIV-1 HXB2R bases 682-705, 800-822, 1307-1337, 1306-1330, 1315-1340, 1395-1425, 1510-1535, 1549-1572, 1743-1771, 1972-1989, 2868-2889, 3008-3042, 3092-3124, 3209-3235, 4052-4079, 4176-4209, 4169-4206, 4394-4428, 4756-4778, 4835-4857, 4952-4969, 5834-5860, 5979-5999, 9431-9457, 9529-9555, 449-473, 550-577, 578-601, 579-600, 624-646, and 680-703.

Specifically, such amplification oligonucleotides consist, comprise, or consist essentially of those selected from (reading 5' to 3'):

- (X) CTCGACGCAGGACTCGGCTTGCTG (SEQ. ID. NO. 19),
- (X) CTCCCCCGCTTAATACTGACGCT (SEQ. ID. NO. 20),
- (X) GGCAAATGGTACATCAGGCCATATCACCTAG (SEQ. ID. NO. 21),
- (X) GGGGTGGCTCCTTCTGATAATGCTG (SEQ. ID. NO. 22),
- (X) CAGAAGGAGCCACCCCAAGATTTA (SEQ. ID. NO. 23),
- (X) GACCATCAATGAGGAAGCTGCAGAATG (SEQ. ID. NO. 24),
- (X) CCCATTCTGCAGCTTCCTCATTGAT (SEQ. ID. NO. 25),
- (X) AGTGACATAGCAGGAATA (SEQ. ID. NO. 26),
- (X) CCATCCTATTTGTTCTGAAGGGTAC (SEQ. ID. NO. 27),
- (X) AGATTTCTCCTACTGGGATAGGT (SEQ. ID. NO. 28),
- (X) GAAACCTTGTTGAGTCCAAAATGCGAACCC (SEQ. ID. NO. 29),
- (X) TGTGCCCTTCTTTGCCAC (SEQ. ID. NO. 30),

- (X) CAGTACTGGATGTGGGTGATGC (SEQ. ID. NO. 31),
 (X) GTCATGCTACTTTGGAATATTTCTGGTGATCCTTT (SEQ. ID. NO. 32),
 (X) CAATACATGGATGATTTGTATGTAGGATCTGAC (SEQ. ID. NO. 33),
 (X) ACCAAAGGAATGGAGGTTCTTTCTGATG (SEQ. ID. NO. 34),
 5 (X) GCATTAGGAATCATTCAAGCACAACCAG (SEQ. ID. NO. 35),
 (X) GCACTGACTAATTTATCTACTTGTTTCATTCCTC (SEQ. ID. NO. 36),
 (X) GGGATTGGAGGAAATGAACAAGTAGATAAATTAGTCAG (SEQ. ID. NO. 37),
 (X) TGTGTACAATCTAGTTGCCATATTCCTGGACTACA (SEQ. ID. NO. 38),
 10 (X) CAAATGGCAGTATTCATCCACA (SEQ. ID. NO. 39),
 (X) GTTTGTATGCTCTGTTGCTATTAT (SEQ. ID. NO. 40),
 (X) CCCTTCACCTTTCCAGAG (SEQ. ID. NO. 41),
 (X) GAGCCCTGGAAGCATCCAGGAAGTCAG (SEQ. ID. NO. 42),
 (X) CTTCTGCTGCTGTCTCCGCTTC (SEQ. ID. NO. 43),
 15 (X) CAAGGGACTTTCCGCTGGGGACTTTCC (SEQ. ID. NO. 44),
 (X) GTCTAACCAGAGAGACCCAGTACAGGC (SEQ. ID. NO. 45),
 (X) GTA CTGGGTCTCTCTGGTTAGACCA (SEQ. ID. NO. 46),
 (X) CACACAACAGACGGGCACACACTACTTG (SEQ. ID. NO. 47),
 (X) CTGAGGGATCTCTAGTTACCAGAGT (SEQ. ID. NO. 48),
 20 (X) CTCTGGTAACTAGAGATCCCTCA (SEQ. ID. NO. 49),
 (X) GTTCGGGCGCCACTGCTAGAGAT (SEQ. ID. NO. 50),
 (X) GCAAGCCGAGTCCTGCGTCGAGA (SEQ. ID. NO. 51)

and the RNA equivalents thereto (SEQ. ID. Nos. 99-131).

Where (X) is nothing or a 5' oligonucleotide sequence that
 25 is recognized by an enzyme, including but not limited to
 the promoter sequence for T7, T3, or SP6 RNA polymerase,
 which enhances initiation or elongation of RNA
 transcription by an RNA polymerase. One example of X
 includes the sequence SEQ. ID. NO. 52:

30 5'-AATTTAATACGACTCACTATAGGGAGA-3'.

These amplification oligonucleotides are used in a
 nucleic acid amplification assay such as the polymerase
 chain reaction or an amplification reaction using RNA
 polymerase, DNA polymerase and RNase H or its equivalent,
 35 as described by Kacian and Fultz, *supra*, and by Sninsky et
 al. US. Patent No. 5,079,351, both hereby incorporated by
 reference herein.

The amplification oligonucleotides and probes of this invention offer a rapid, non-subjective method of identification and quantitation of a sample for specific sequences unique to strains of Human Immunodeficiency Virus type 1.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

Description of the Preferred Embodiments

We have discovered particularly useful DNA probes complementary to particular nucleic acid sequences of Human Immunodeficiency Virus type 1. Furthermore, we have successfully used these probes in a specific assay for the detection of Human Immunodeficiency Virus type 1, distinguishing it from the known and presumably most closely related taxonomic or phylogenetic neighbors found in human blood or tissues.

We have also identified particularly useful amplification oligonucleotides which are complementary to the Human Immunodeficiency Virus type 1 nucleic acid, and have used these oligonucleotides, e.g., as primers or promoter primer combinations (i.e., a primer having a promoter sequence attached), to amplify the nucleic acid of Human Immunodeficiency Virus, allowing its direct detection in a sample.

Useful guidelines for designing amplification oligonucleotides and probes with desired characteristics are described herein. The optimal sites for amplifying and probing contain two, and preferably three, conserved regions greater than about 15 bases in length, within about 350 bases, and preferably within 150 bases, of contiguous sequence. The degree of amplification observed with a set of primers or promotor/primers depends on several factors, including the ability of the oligonucleotides to hybridize to their complementary sequences and their ability to be extended enzymatically. Because

the extent and specificity of hybridization reactions are affected by a number of factors, manipulation of those factors will determine the exact sensitivity and specificity of a particular oligonucleotide, whether perfectly
5 complementary to its target or not. The importance and effect of various assay conditions are known to those skilled in the art as described in Hogan et al., EPO Patent Application No. PCT/US87/03009, entitled "Nucleic Acid Probes for Detection and/or Quantitation of Non-Viral
10 Organisms"; and Milliman, entitled "Nucleic Acid Probes to Haemophilus influenzae," U.S. Serial No. 07/690,788, filed 4/25/91 assigned to the same assignee as the present application and hereby incorporated by reference herein.

The length of the target nucleic acid sequence and,
15 accordingly, the length of the probe sequence can be important. In some cases, there may be several sequences from a particular region, varying in location and length, which will yield probes with the desired hybridization characteristics. In other cases, one sequence may be
20 significantly better than another which differs merely by a single base. While it is possible for nucleic acids that are not perfectly complementary to hybridize, the longest stretch of perfectly homologous base sequence will normally primarily determine hybrid stability. While
25 oligonucleotide probes of different lengths and base composition may be used, oligonucleotide probes preferred in this invention are between about 10 to 50 bases in length and are sufficiently homologous to the target nucleic acid to hybridize under stringent hybridization
30 conditions. We have found that optimal primers have target-binding regions of 18-38 bases, with a predicted T_m (melting temperature) to target of about 65°C.

Amplification oligonucleotides or probes should be positioned so as to minimize the stability of the
35 oligomer:nontarget (i.e., nucleic acid with similar sequence to target nucleic acid) nucleic acid hybrid. It is preferred that the amplification oligomers and

detection probes are able to distinguish between target and non-target sequences. In designing probes, the differences in these T_m values should be as large as possible (e.g., at least 2°C and preferably 5°C).

5 Regions of the nucleic acid which are known to form strong internal structures inhibitory to hybridization are less preferred. Examples of such structures include hairpin loops. Likewise, probes with extensive self-complementarity should be avoided.

10 The degree of non-specific extension (primer-dimer or non-target copying) can also affect amplification efficiency, therefore primers are selected to have low self- or cross- complementarity, particularly at the 3' ends of the sequence. Long homopolymer tracts and high GC
15 content are avoided to reduce spurious primer extension. Commercial computer programs are available to aid in this aspect of the design. Available computer programs include MacDNASIS™ 2.0 (Hitachi Software Engineering American Ltd.) and OLIGO® ver. 4.1 (National Bioscience).

20 Hybridization is the association of two single strands of complementary nucleic acid to form a hydrogen bonded double strand. It is implicit that if one of the two strands is wholly or partially involved in a hybrid that it will be less able to participate in formation of
25 a new hybrid. By designing a probe so that a substantial portion of the sequence of interest is single stranded, the rate and extent of hybridization may be greatly increased. If the target is an integrated genomic sequence then it will naturally occur in a double stranded
30 form, as is the case with the product of the polymerase chain reaction (PCR). These double-stranded targets are naturally inhibitory to hybridization with a probe and require denaturation prior to the hybridization step. Finally, there can be intramolecular and intermolecular
35 hybrids formed within a probe if there is sufficient self complementarity. Such structures can be avoided through careful probe design. Commercial computer programs are

available to search for this type of interaction. Available computer programs include MacDNASIS™ 2.0 (Hitachi Software Engineering American Ltd.) and OLIGO® ver. 4.1 (National Bioscience).

5 Once synthesized, selected oligonucleotide probes may be labelled by any of several well known methods. 2 J. Sambrook, E.F. Fritsch and T. Maniatis, Molecular Cloning 11 (2d ed. 1989). Useful labels include radio-isotopes as well as non-radioactive reporting groups. We
10 currently prefer to use acridinium esters.

Oligonucleotide/target hybrid melting temperature may be determined by isotopic methods well known to those skilled in the art. It should be noted that the T_m for a given hybrid will vary depending on the hybridization
15 solution being used. Sambrook, et al. supra.

Rate of hybridization may be measured by determining the C_0t_x . The rate at which a probe hybridizes to its target is a measure of the thermal stability of the target secondary structure in the probe region. The standard
20 measurement of hybridization rate is the C_0t_x which is measured as moles of nucleotide per liter times seconds. Thus, it is the concentration of probe times the time at which 50% of maximal hybridization occurs at that concentration. This value is determined by hybridizing
25 various amounts of probe to a constant amount of target for a fixed time. The C_0t_x is found graphically by standard procedure.

The following examples set forth oligonucleotide probes complementary to a unique nucleic acid sequence
30 from a target organism, and their use in a hybridization assay.

Examples:

Probes specific for Human Immunodeficiency Virus type 1 were identified by comparison of sequences obtained from
35 the published database GenBank. Sequences ID Nos. 1-12 were characterized and shown to be specific for Human

Immunodeficiency Virus type 1. Phylogenetically near neighbors including Human Immunodeficiency Virus type 2, Human T-cell Leukemia Virus type 1 and Human T-Cell Leukemia Virus type 2 were used as comparisons with the sequence of Human Immunodeficiency Virus Type 1.

Example 1. Probes for HIV

A hybridization protection assay was used to demonstrate the reactivity and specificity of the probes for Human Immunodeficiency Virus type 1. The probes were first synthesized with a non-nucleotide linker, then labelled with a chemiluminescent acridinium ester (AE) as described by Arnold, et al., PCT/US88/03361, entitled "Acridinium Ester Labeling and Purification of Nucleotide Probes," hereby incorporated by reference herein. The acridinium ester attached to an unhybridized probe is susceptible to hydrolysis and rendered non-chemiluminescent under mild alkaline conditions. However, the acridinium ester attached to hybridized probe is relatively resistant to hydrolysis. Thus, it is possible to assay for hybridization of acridinium ester-labelled probe by incubation with an alkaline buffer, followed by detection of chemiluminescence in a luminometer. Results are given in Relative Light Units (RLU); the quantity of photons emitted by the labelled-probe measured by the luminometer.

In the following experiment, DNA prepared from clones containing full or partial sequences of the target viruses was assayed. An example of a method for preparing the DNA from clones is provided by Sambrook et al, supra. The source of DNA for the clones was as follows; Human Immunodeficiency Virus type 1, BH10 (L. Ratner et al., Nature 312:277-284. 1985); Human Immunodeficiency Virus type 2 NIHZ (J.F. Zagury, et al., Proc. Natl. Acad. Sci. USA 85:5941-5945. 1988), Human T-cell leukemia virus type 1 pMT-2, (M. Clarke et al. Nature 305:60-62. 1983); Human T-cell leukemia virus type 2 (K. Shimotohmo et al. Proc.

Natl. Acad. Sci. USA 82:3101-3105. 1985); and Human Hepatitis B Virus serotype ADW, obtained from ATCC(# 45020). Target in 50 μ l of 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10 mM ethylenediaminetetraacetic acid (EDTA), 1% lithium lauryl sulfate, pH 7.4, was denatured at 95°C for 5 min, cooled on wet ice, and 0.04 pmol of probe in 50 μ l of 0.1 M lithium succinate buffer, pH 4.7, 2% (w/v) lithium lauryl sulfate, 1.2 M lithium chloride, 10 mM EDTA and 20 mM ethyleneglycol-bis-(beta-aminoethyl ether) N,N,N',N'-tetraacetic acid (EGTA) was added. Hybridization was carried out at 60°C for 10 min, followed by addition of 300 μ l of 0.6 M sodium borate pH 8.5, 1% Triton X-100 and a second incubation at 60°C for 6 min to hydrolyze the AE on unhybridized probe. Samples were cooled in ice water for 1 min, placed at room temperature for another 3 min, and then analyzed in a LEADER 1 luminometer equipped with automatic injection of detection reagent I (containing 0.1% hydrogen peroxide and 1 mM nitric acid) and detection reagent II (containing 1 N sodium hydroxide and a surfactant component). Some of the hybridization reactions were enhanced with the addition of 4 pmol of unlabelled "helper probe" as disclosed in Hogan et al., U.S. Patent No. 5,030,557 entitled "Means and Methods for Enhancing Nucleic Acid Hybridization", hereby incorporated by reference herein. An RLU value greater than 5,000 RLU was a positive result; less than 5,000 RLU was a negative result.

The following data (Table 1) show that the probes do not cross react with viral DNA from closely related viruses found in human blood or tissues. The samples also gave a positive signal when tested with a probe specific to each target, thereby confirming sample adequacy.

Table 1. Hybridization Assay with HIV-1 probes.

	Target:	1	2	3	4	5	6	7
	Probe							
	Sequence							
5	ID No:							
	1	179,166	482	500	496	190	496	470
	2	14,992	563	394	377	409	383	465
	4	61,691	2,818	750	695	686	702	642
	6	28,038	546	408	375	356	369	372
10	7	27,407	640	401	252	366	343	359
	8	45,868	1,432	395	392	386	401	404
	9	15,971	721	280	268	261	274	284
	10	59,007	714	264	280	284	272	567
	11	25,856	4,641	3,598	3,736	3,711	3,855	3,388
15	12	140,691	1,846	602	694	531	534	1,236

Target 1 = HIV-1 BH10 isolate 9 Kb SstI fragment, Target 2 = Human Immunodeficiency Virus Type 2 (NIHZ isolate) 9 Kb NaeI fragment, Target 3 = Human T-cell leukemia virus type 1 (pMT-2) 5' 4.6 Kb SstI-BamHI fragment; Target 4 = Human T-cell leukemia virus type 1 3' 4.4 Kb XbaI-SstI fragment, Target 5 = Human T-cell leukemia virus type 2 3.5 Kb BamHI fragment, Target 6 = Human T-cell leukemia virus type 2 5 Kb BamHI fragment, Target 7 = Human Hepatitis B virus serotype ADW 1.3, 1.8 Kb BamHI fragments.

The above data confirm that the novel probes herein disclosed and claimed are capable of distinguishing Human Immunodeficiency Virus type 1 from these viruses found in human blood.

30 Example 2. Amplification of HIV by PCR

To demonstrate the reactivity of the primers and probes for Human Immunodeficiency Virus type 1, the following experiment was performed. Zero, 20, or 100 copies of plasmid DNA containing Human Immunodeficiency Virus DNA was linearized with a restriction endonuclease, and added to amplification reactions containing 50 pmol of

15

each primer, 10 mM Tris HCl pH 8, 50 mM KCl, 1.25 mM MgCl₂, 0.25 mM each of dATP, dTTP, dCTP, dGTP, and 2.5 U Taq DNA polymerase in 50 μ l. The reactions were incubated at 95°C for 1-2 min, and then cycled 35 times at 55°C for 15 sec, 72°C for 30 sec, and 95°C for 20 sec in a Perkin-Elmer 9600 thermocycler or 55°C for 30 sec, 72°C for 60 sec, and 95°C for 60 sec in a Perkin-Elmer 48 well thermocycler. Following cycling, the reactions were incubated at 72°C for 6-7 min and stored at 4°C. Ten μ l of the product was analyzed by hybridization protection assay with 0.04 pmol of labeled probe. The data are shown in Table 2. RLU greater than 7,000 is considered a positive result.

Table 2. Amplification of Human Immunodeficiency Virus Type 1 by PCR

15	Primer Sequence ID Nos:	Probe Sequence ID. No.	Sample		
			0 c	20 c	RLU 100 c
20	19/20*	1	886	827,202	723,008
	21/22*	2	2,677	24,030	48,521
	*23/25	3	370	144,082	603,456
	*24/27	4	4,042	81,052	163,355
	*26/28	5	263	273,023	593,022
25	*29/30	6	1,008	328,736	366,590
	*31/32	7	3,394	73,690	86,168
	*33/34	8	1,648	7,152	24,027
	*35/36	9	560	82,980	145,681
	*39/40	10	810	279,079	299,815
30	*39/41	10	886	362,914	427,500
	42/43*	11	5,830	680,788	1,939,372
	*44/45	12	1,387	21,428	130,709

The starred (*) primers had the sequence 5'-AATTTAATACGACTCACTATAGGGAGA-3' attached to the 5' end of the primer. 0 c = 0 copies of HIV DNA, 20 c = 20 copies of HIV DNA, 100 c = 100 copies of HIV DNA. Probe 1 was

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used in the presence of unlabeled helper probe SEQ. ID. No. 15. Probe 7 was used in the presence unlabeled helper probe SEQ. ID. No. 16. Probe 10 was used in the presence of unlabeled helper probe SEQ. ID. No. 17. Probe 12 was
 5 used in the presence of unlabeled helper probe SEQ. ID. No. 18. As the copy number increased, RLU increased. Thus, the primers of the present invention were able to successfully amplify, by PCR, HIV type 1 target sequences which were detected using the probes of the present
 10 invention.

Example 3. Patient samples

In this example, patient samples containing lysate prepared from 200,000 Ficoll-Hypaque purified white blood cells from individuals known to be infected with HIV type
 15 1 or an individual not infected with HIV type 1 (negative) were analyzed as described in Example 2. These cells were prepared as described in Ryder and Kacian, entitled "Preparation of nucleic acid from blood," U.S. Serial number 07/898,785, filed 6/12/92. The results are shown
 20 in Table 3.

Table 3. PCR Assay

		Sample		RLU	
		Patient 1	Patient 2	Negative	
Primer	Probe				
25 Sequence	Sequence				
ID Nos:	ID. No.				
19/20*	1	27,616	71,981	886	
21/22*	2	34,949	35,483	565	
*23/25	3	45,230	93,529	455	
30 *24/27	4	2,355	25,329	1,052	
*26/28	5	22,345	26,014	369	
*31/32	7	200,418	130,486	481	
*33/34	8	43,993	40,389	705	
*39/40	10	36,310	50,838	976	
35 *39/41	10	55,582	98,504	993	

Table 3. PCR Assay (Cont'd)

		Sample	RLU	
		Patient 1	Patient 2	Negative
Primer	Probe			
5 Sequence	Sequence			
ID Nos:	ID. No.			
42/43*	11	99,028	207,605	6,057
*44/45	12	55,082	80,388	1,496

The starred (*) primers had the sequence 5'-

10 AATTTAATACGACTCACTATAGGGAGA-3' attached to the 5' end of the primer. The primers of the present invention were able to amplify by PCR HIV type 1 target sequences present in individuals infected with HIV. The amplified target sequences were detected by the probes of the present

15 invention. Thus, individuals containing HIV type 1 and an individual not containing HIV type 1 were correctly identified.

Example 4. Non-PCR Amplification

To show that the amplification oligomers also work in

20 a transcription based amplification assay, 0, 2,000, or 20,000 copies of plasmid DNA containing HIV type 1 was linearized using a restriction endonuclease, and heated to 95°C for two min and cooled to 37°C for 1 min. Following addition of 800 U of MMLV reverse transcriptase the

25 reactions were incubated for 12 min at 37°C, heated to 95°C for two min, and cooled to 37°C for one min. 800 U of MMLV reverse transcriptase and 400 U of T7 RNA polymerase were added and the reactions were incubated for 3 hr at 37°C. The final amplification conditions were 70

30 mM Tris HCl, pH 8, 35 mM KCl, 15 mM KOH neutralized N-acetyl-cysteine, 6 mM rGTP, 4 mM rCTP, 4 mM rATP, 4 mM rUTP, 1 mM each of dTTP, dATP, dCTP and dGTP, and 22 mM MgCl₂ in 100 µl. Ten µl of each reaction was mixed with 40 µl of water and assayed as described for Table 1 except

35 that the hybridization buffer contained 20 mM aldrithiol. The results in RLU are shown in Table 4.

Table 4. Transcription-Based Amplification Assay

		RLU		
Primers	Probe	0 c	2,000 c	20,000 c
Sequence	Sequence			
5 ID Nos:	ID. No.			
19/20*	1	681	24,170	190,536
21/22*	2	793	62,476	523,770
*23/25	3	2,239	812,577	1,126,045
*24/27	4	1,901	160,274	780,351
10 *26/28	5	2,555	877,893	1,167,756
*29/30	6	868	299,255	880,119
*31/32	7	871	129,732	969,034
*33/34	8	710	134,887	986,266
*35/36	9	884	128,981	1,021,865
15 *39/40	10	1,597	375,629	478,883
*39/41	10	1,264	499,304	495,509
*44/45	12	2,426	41,684	542,339

The starred (*) primers had the sequence 5'-AATTTAATACGACTCACTATAGGGAGA-3' attached to the 5' end of the primer. Probe 1 was used in the presence of unlabelled helper probe SEQ. ID. No. 15. Probe 7 was used in the presence of unlabelled helper probe SEQ. ID. No. 16, probe 10 was used in the presence of unlabelled helper probe SEQ. ID. No. 17, and probe 12 was used in the presence of unlabelled helper probe SEQ. ID. No. 18. 0 c = 0 copies of HIV DNA, 2,000 c = 2,000 copies of HIV DNA, 20,000 c = 20,000 copies of HIV DNA.

As the copy number increased RLU also increased. Thus, the primers of the present invention can be used to amplify HIV type 1 target sequences using a transcription based amplification assay and the amplified target sequences can be detected using the probes of the present invention.

Example 5.

This example demonstrates the ability of probes for Human Immunodeficiency Virus type 1 to detect low levels

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of target oligomer produced in a transcription based amplification assay. Zero or 10 copies of plasmid DNA containing HIV type I sequence was linearized using a restriction endonuclease, heated in the presence of 1 μ g of human DNA to 95°C for eight minutes, and then cooled to 42°C for six minutes. Amplification was carried out at 42°C for two hours using 800 U MMLV reverse transcriptase and 400 U of T7 RNA polymerase, in the following reaction mix: 58 mM Tris HCl pH 8, 17.5 mM MgCl₂, 0.05 mM zinc acetate, 10% glycerol, 6.25 mM rGTP, 2.5 mM rCTP, 6.25 mM rATP, 2.5 mM rUTP, 0.2 mM dTTP, 0.2 mM dATP, 0.2 mM, 0.2 mM dCTP and 0.2 mM dGTP. Primer SEQ ID NOs. 26, 28, and 41 were used at a concentration of 30 pmol, primer SEQ ID NO. 39 was used at a concentration of 15 pmol. The entire reaction was analyzed using the hybridization protection assay with 0.04 pmol of probe in 100 μ l of the hybridization buffer (supplemented with 20 mM aldrithiol) as described in Example 1. Probe SEQ ID NO. 10 was hybridized in the presence of 2 pmol unlabeled helper SEQ ID NO. 17.

Table 5. Low Level Transcription-Based Amplification Assay

Primers SEQ ID NOS.	Probe SEQ	RLU	
		0 copies	10 copies
*26/28	5	1,293	64,639
*39/40	10	2,143	564,185

The 10 copy values represent the average of ten replicates. The starred (*) primers had the sequence 5'-AATTTAATACGACTCACTATAGGGAGA-3' attached to the 5' end of the primer.

Other embodiments are within the following claims.

20

(1) GENERAL INFORMATION:

(i) APPLICANT: Sherrol H. McDonough, Thomas B. Ryder,
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(ii) TITLE OF INVENTION: NUCLEIC ACID AMPLIFICATION
OLIGONUCLEOTIDES AND PROBES
TO HUMAN IMMUNODEFICIENCY
VIRUS TYPE 1

(iii) NUMBER OF SEQUENCES: 140

(iv) CORRESPONDENCE ADDRESS:

10 (A) ADDRESSEE: Lyon & Lyon
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(D) STATE: California
(E) COUNTRY: USA
15 (F) ZIP: 90017

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb storage
(B) COMPUTER: IBM PS/2 Model 50Z or 55SX
(C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
20 (D) SOFTWARE: WordPerfect (Version 5.0)

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

25 (vii) PRIOR APPLICATION DATA:

Prior applications total, including application described
below: 2

(A) APPLICATION NUMBER: U.S. Serial No. 07/550,837
(B) FILING DATE: 7/10/90
30 (A) APPLICATION NUMBER: U.S. Serial No. 07/379,501
(B) FILING DATE: 7/11/89

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5 (2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
10 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

GACTAGCGGA GGCTAGAAGG AGAGAGATGG G 31

(3) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 2:

GAAGGCTTTC AGCCCAGAAG TAATACCCAT G 31

(4) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 3:

ATTGTCATGG CTGCTTGATG TCCCCCCT 30

30 (5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 4:

CTTCCCCTTG GTTCTCTCAT CTGGCC 26

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(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 5:

GTCATCCATC CTATTGTTC CTGAAGGGTA CTAGTAG 37

(7) INFORMATION FOR SEQ ID NO: 6:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 6:

CTCCCTGACA TGCTGTCATC ATTTCTTCTA GTG 33

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 7:

GTGGAAGCAC ATTGTACTGA TATCTAATCC C 31

25 (9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 8:

GCTCCTCTAT TTTTGTTCCTA TGCTGCCCTA TTTCTAA 37

(10) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

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- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 9:
CCTTTGTGTG CTGGTACCCA TGC 23
- (11) INFORMATION FOR SEQ ID NO: 10:
- 5 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 10 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 10:
CTACTATTCT TTCCCCTGCA CTGTACCCC 29
- (12) INFORMATION FOR SEQ ID NO: 11:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 29
- 15 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 11:
AAAGCCTTAG GCATCTCCTA TGGCAGGAA 29
- 20 (13) INFORMATION FOR SEQ ID NO: 12:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 25 (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 12:
GCAGCTGCTT ATATGCAGGA TCTGAGGG 28
- (14) INFORMATION FOR SEQ ID NO: 13:
- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 33
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 13:
35 CAAGGCAAGC TTTATTGAGG CTTAAGCAGT GGG 33
- (15) INFORMATION FOR SEQ ID NO: 14:
- (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 14:
ATCTCTAGCA GTGGCGCCCG AACAGGGA 28

(16) INFORMATION FOR SEQ ID NO: 15:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 15:
TGCGAGAGCG TCAGTATTAA GCGG 24

15 (17) INFORMATION FOR SEQ ID NO: 16:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 16:
CTACTTTGGA ATATTGCTGG TGATCCTTTC CATCCC 36

(18) INFORMATION FOR SEQ ID NO: 17:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 17:
30 CCAATCCCCC CTTTCTTTT AAAATTGTGG ATG 33

(19) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 18:
CTCGCCACTC CCCAGTCCCG CCCA 24
- (20) INFORMATION FOR SEQ ID NO: 19:
- (i) SEQUENCE CHARACTERISTICS:
- 5 (A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 19:
10 CTCGACGCAG GACTCGGCTT GCTG 24
- (21) INFORMATION FOR SEQ ID NO: 20:
- (i) SEQUENCE CHARACTERISTICS:
- 15 (A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 20:
CTCCCCCGCT TAATACTGAC GCT 23
- (22) INFORMATION FOR SEQ ID NO: 21:
- 20 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 21:
25 GGCAAATGGT ACATCAGGCC ATATCACCTA G 31
- (23) INFORMATION FOR SEQ ID NO: 22:
- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 22:
GGGGTGGCTC CTTCTGATAA TGCTG 25
- 35 (24) INFORMATION FOR SEQ ID NO: 23:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 23:

CAGAAGGAGC CACCCACAA GATTTA 26

(25) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 24:

15 GACCATCAAT GAGGAAGCTG CAGAATG 27

(26) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 25:

CCCATTCTGC AGCTTCCTCA TTGAT 25

(27) INFORMATION FOR SEQ ID NO: 26:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 26:

AGTGACATAG CAGGAACTA 19

(28) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 27:
CCATCCTATT TGTTCCTGAA GGTAC 26

(29) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 28:

10 AGATTTCTCC TACTGGGATA GGT 23

(30) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30
(B) TYPE: nucleic acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 29:

GAAACCTTGT TGAGTCCAAA ATGCGAACCC 30

(31) INFORMATION FOR SEQ ID NO: 30:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 30:

TGTGCCCTTC TTTGCCAC 18

(32) INFORMATION FOR SEQ ID NO: 31:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22
30 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 31:

CAGTACTGGA TGTGGGTGAT GC 22

35 (33) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 32:
GTCATGCTAC TTTGGAATAT TTCTGGTGAT CCTTT 35
(34) INFORMATION FOR SEQ ID NO: 33:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 33:
CAATACATGG ATGATTTGTA TGTAGGATCT GAC 33
15 (35) INFORMATION FOR SEQ ID NO: 34:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 34:
ACCAAAGGAA TGGAGGTTCT TTCTGATG 28
(36) INFORMATION FOR SEQ ID NO: 35:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 35:
30 GCATTAGGAA TCATTCAAGC ACAACCAG 28
(37) INFORMATION FOR SEQ ID NO: 36:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 36:

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GCACTGACTA ATTTATCTAC TTGTTCA TTT CCTC 34

(38) INFORMATION FOR SEQ ID NO: 37:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 38
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 37:

GGGATTGGAG GAAATGAACA AGTAGATAAA TTAGTCAG 38

10 (39) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
15 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 38:

TGTGTACAAT CTAGTTGCCA TATTCCTGGA CTACA 35

(40) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 22
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 39:

25 CAAATGGCAG TATTCATCCA CA 22

(41) INFORMATION FOR SEQ ID NO: 40:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
30 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 40:

GTTTGTATGT CTGTTGCTAT TAT 23

(42) INFORMATION FOR SEQ ID NO: 41:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18
(B) TYPE: nucleic acid

30

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 41:

CCCTTCACCT TTCCAGAG 18

5 (43) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

10 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 42:

GAGCCCTGGA AGCATCCAGG AAGTCAG 27

(44) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 21

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 43:

20 CTTCGTCGCT GTCTCCGCTT C 21

(45) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: nucleic acid

25 (C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 44:

CAAGGGACTT TCCGCTGGGG ACTTTCC 27

(46) INFORMATION FOR SEQ ID NO: 45:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

35 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 45:

GTCTAACCAG AGAGACCCAG TACAGGC 27

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- (47) INFORMATION FOR SEQ ID NO: 46:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- 5 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 46:
- GTACTGGGTC TCTCTGGTTA GACCA 25
- (48) INFORMATION FOR SEQ ID NO: 47:
- 10 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 28
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 15 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 47:
- CACACAACAG ACGGGCACAC ACTACTTG 28
- (49) INFORMATION FOR SEQ ID NO: 48:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25
- 20 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 48:
- CTGAGGGATC TCTAGTTACC AGAGT 25
- 25 (50) INFORMATION FOR SEQ ID NO: 49:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 23
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 30 (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 49:
- CTCTGGTAAC TAGAGATCCC TCA 23
- (51) INFORMATION FOR SEQ ID NO: 50:
- (i) SEQUENCE CHARACTERISTICS:
- 35 (A) LENGTH: 23
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 50:
GTTTCGGGCGC CACTGCTAGA GAT 23

(52) INFORMATION FOR SEQ ID NO: 51:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 51:
GCAAGCCGAGT CCTGCGTCG AGA 23

(53) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27

15 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 52:
AATTTAATAC GACTCACTAT AGGGAGA 27

20 (54) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 53:
CCCATCTCTC TCCTTCTAGC CTCCGCTAGT C 31

(55) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 54:
35 CATGGGTATT ACTTCTGGGC TGAAAGCCTT C 31

(56) INFORMATION FOR SEQ ID NO: 55:

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- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
5 (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 55:
AGTGGGGGGA CATCAAGCAG CCATGCAAAT 30
- (57) INFORMATION FOR SEQ ID NO: 56:
- (i) SEQUENCE CHARACTERISTICS:
10 (A) LENGTH: 26
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 56:
15 GGCCAGATGA GAGAACCAAG GGGAAG 26
- (58) INFORMATION FOR SEQ ID NO: 57:
- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37
(B) TYPE: nucleic acid
20 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 57:
CTACTAGTAC CCTTCAGGAA CAAATAGGAT GGATGAC 37
- (59) INFORMATION FOR SEQ ID NO: 58:
- 25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- 30 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 58:
CACTAGAAGA AATGATGACA GCATGTCAGG GAG 33
- (60) INFORMATION FOR SEQ ID NO: 59:
- (i) SEQUENCE CHARACTERISTICS:
35 (A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 59:
GGGATTAGAT ATCAGTACAA TGTGCTTCCA C 31

(61) INFORMATION FOR SEQ ID NO: 60:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 60:

10 TTAGAAATAG GGCAGCATAG AACAAAAATA GAGGAGC 37

(62) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 61:

GCATGGGTAC CAGCACACAA AGG 23

(63) INFORMATION FOR SEQ ID NO: 62:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 62:

GGGGTACAGT GCAGGGGAAA GAATAGTAG 29

(64) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 63:

TTCCTGCCAT AGGAGATGCC TAAGGCTTT 29

35 (65) INFORMATION FOR SEQ ID NO: 64:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 64:
CCCTCAGATC CTGCATATAA GCAGCTGC 28
(66) INFORMATION FOR SEQ ID NO: 65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 65:
CCCCTGCTT AAGCCTCAAT AAAGCTTGCC TTG 33
15 (67) INFORMATION FOR SEQ ID NO: 66:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 66:
TCCCTGTTCG GCGCCACTG CTAGAGAT 28
(68) INFORMATION FOR SEQ ID NO: 67:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 67:
30 GACUAGCGGA GGCUAGAAGG AGAGAGAUGG G 31
(69) INFORMATION FOR SEQ ID NO: 68:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 68:
GAAGGCUUUC AGCCCAGAAG UAAUACCCAU G 31

(70) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 30
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 69:
10 AUUUGCAUGG CUGCUUGAUG UCCCCCACU 30

(71) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26
(B) TYPE: nucleic acid
15 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 70:
CUUCCCCUUG GUUCUCUCAU CUGGCC 26

(72) INFORMATION FOR SEQ ID NO: 71:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 71:
GUCAUCCAUC CUAUUUGUUC CUGAAGGGUA CUAGUAG 37

(73) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
30 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 72:
CUCCCUGACA UGCUGUCAUC AUUUCUUCUA GUG 33

35 (74) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 73:

5 GUGGAAGCAC AUUGUACUGA UAUCUAAUCC C 31

(75) INFORMATION FOR SEQ ID NO: 74:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 74:

GCUCCUCUAU UUUUGUUCUA UGCUGCCCUA UUUCUAA 37

(76) INFORMATION FOR SEQ ID NO: 75:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 75:

CCUUUGUGUG CUGGUACCCA UGC 23

(77) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 76:

CUACUAUUCU UCCCCUGCA CUGUACCCC 29

30 (78) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 77:

AAAGCCUAG GCAUCUCCUA UGGCAGGAA 29

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(79) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 78:

GCAGCUGCUU AUAUGCAGGA UCUGAGGG 28

(80) INFORMATION FOR SEQ ID NO: 79:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 79:

CAAGGCAAGC UUUAUUGAGG CUUAAGCAGU GGG 33

(81) INFORMATION FOR SEQ ID NO: 80:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 80:

AUCUCUAGCA GUGGCGCCCG AACAGGGA 28

25 (82) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 81:

CCCAUCUCUC UCCUUCUAGC CUCCGCUAGU C 31

(83) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 82:
CAUGGGUAUU ACUUCUGGGC UGAAAGCCUU C 31

(84) INFORMATION FOR SEQ ID NO: 83:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 83:
AGUGGGGGGA CAUCAAGCAG CCAUGCAA U 31

(85) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

15 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 84:
GGCCAGAUGA GAGAACCAAG GGGAAG 26

20 (86) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 85:
CUACUAGUAC CCUUCAGGAA CAAUAGGAU GGAUGAC 37

(87) INFORMATION FOR SEQ ID NO: 86:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 33

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 86:
35 CACUAGAAGA AAUGAUGACA GCAUGUCAGG GAG 33

(88) INFORMATION FOR SEQ ID NO: 87:

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(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 87:

GGGAUUAGAU AUCAGUACAA UGUGCUUCCA C 31

(89) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 88:

15 UUAGAAUAG GGCAGCAUAG AACAAAAUA GAGGAGC 37

(90) INFORMATION FOR SEQ ID NO: 89:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

20

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 89:

GCAUGGGUAC CAGCACACAA AGG 23

(91) INFORMATION FOR SEQ ID NO: 90:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

30

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 90:

GGGGUACAGU GCAGGGGAAA GAAUAGUAG 29

(92) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 91:
UUCCUGCCAU AGGAGAUGCC UAAGGCUUU 29
- (93) INFORMATION FOR SEQ ID NO: 92:
- (i) SEQUENCE CHARACTERISTICS:
- 5 (A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 92:
10 CCCUCAGAU CUGCAUAUAA GCAGCUGC 28
- (94) INFORMATION FOR SEQ ID NO: 93:
- (i) SEQUENCE CHARACTERISTICS:
- 15 (A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 93:
CCCACUGCUU AAGCCUCAU AAAGCUUGCC UUG 33
- (95) INFORMATION FOR SEQ ID NO: 94:
- 20 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 38
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 94:
25 UCCCUGUUCG GCGCCACUG CUAGAGAU 38
- (96) INFORMATION FOR SEQ ID NO: 95:
- (i) SEQUENCE CHARACTERISTICS:
- 30 (A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 95:
CCGCTTAATA CTGACGCTCT CGCA 24
- 35 (97) INFORMATION FOR SEQ ID NO: 96:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 36

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 96:
5 GGGATGGAAA GGATCACCAG CAATATTCCA AAGTAG 36
(98) INFORMATION FOR SEQ ID NO: 97:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 97:
CATCCACAAT TTAAAAGAA AAGGGGGGAT TGG 33
(99) INFORMATION FOR SEQ ID NO: 98:
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
20 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 98:
TGGGCGGGAC TGGGGAGTGG CGAG 24
(100) INFORMATION FOR SEQ ID NO: 99:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 99:
CUCGACGCAG GACUCGGCUU GCUG 24
30 (101) INFORMATION FOR SEQ ID NO: 100:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 100:
CUCCCCCGCU UAAUACUGAC GCU 23

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- (102) INFORMATION FOR SEQ ID NO: 101:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 31
- (B) TYPE: nucleic acid
- 5 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 101:
- GGCAAUGGU ACAUCAGGCC AUAUCACCUA G 31
- (103) INFORMATION FOR SEQ ID NO: 102:
- 10 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- 15 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 102:
- GGGGUGGCUC CUUCUGAUAA UGCUG 25
- (104) INFORMATION FOR SEQ ID NO: 103:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 26
- 20 (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 103:
- CAGAAGGAGC CACCCACAA GAUUUA 26
- 25 (105) INFORMATION FOR SEQ ID NO: 104:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 27
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 30 (D) TOPOLOGY: linear
- (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 104:
- GACCAUCAAU GAGGAAGCUG CAGAAUG 27
- (106) INFORMATION FOR SEQ ID NO: 105:
- (i) SEQUENCE CHARACTERISTICS:
- 35 (A) LENGTH: 25
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 105:
CCCAUUCUGC AGCUUCCUCA UUGAU 25

(107) INFORMATION FOR SEQ ID NO: 106:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

10 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 106:
AGUGACAUAG CAGGAACUA 19

(108) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26

15 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 107:
CCAUCCUAUU UGUUCCUGAA GGGUAC 26

20 (109) INFORMATION FOR SEQ ID NO: 108:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 23

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

25 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 108:
AGAUUUCUCC UACUGGGAUA GGU 23

(110) INFORMATION FOR SEQ ID NO: 109:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 30

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 109:
35 GAAACCUUGU UGAGUCCAAA AUGCGAACCC 30

(111) INFORMATION FOR SEQ ID NO: 110:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 110:
UGUGCCCUUC UUUGCCAC 18

(112) INFORMATION FOR SEQ ID NO: 111:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 111:
CAGUACUGGA UGUGGGUGAU GC 22

15 (113) INFORMATION FOR SEQ ID NO: 112:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 112:
GUCAUGCUCAC UUUGGAAUUAU UUCUGGUGAU CCUUU 35

(114) INFORMATION FOR SEQ ID NO: 113:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 113:
30 CAAUACAUGG AUGAUUUGUA UGUAGGAUCU GAC 33

(115) INFORMATION FOR SEQ ID NO: 114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 114:
ACCAAAGGAA UGGAGGUUCU UUCUGAUG 28

(116) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 115:

10 GCAUUAGGAA UCAUUAAGC ACAACCAG 28

(117) INFORMATION FOR SEQ ID NO: 116:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 34
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 116:

GCACUGACUA AUUUAUCUAC UUGUUCAUUU CCUC 34

(118) INFORMATION FOR SEQ ID NO: 117:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 38
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 117:

25 GGGAUUGGAG GAAAUAGAACA AGUAGAUAAA UUAGUCAG 38

(119) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 35
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 118:

UGUGUACAAU CUAGUUGCCA UAUUCCUGGA CUACA 35

35 (120) INFORMATION FOR SEQ ID NO: 119:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22

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(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 119:
5 CAA AUGGCAG UAUUCAUCCA CA 22
(121) INFORMATION FOR SEQ ID NO: 120:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
10 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 120:
GUUUGUAUGU CUGUUGCUAU UAU 23
(122) INFORMATION FOR SEQ ID NO: 121:
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
20 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 121:
CCCUUCACCU UCCAGAG 18
(123) INFORMATION FOR SEQ ID NO: 122:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27
25 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 122:
GAGCCUGGA AGCAUCCAGG AAGUCAG 27
30 (124) INFORMATION FOR SEQ ID NO: 123:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
35 (D) TOPOLOGY: linear
(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 123:
CUUCGUCGU GUCUCCGCU C 21

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(125) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 124:

CAAGGGACUU UCCGCUGGGG ACUUUCC 27

(126) INFORMATION FOR SEQ ID NO: 125:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 27
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

15 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 125:

GUCUAACCAG AGAGACCCAG UACAGGC 27

(127) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 126:

GUACUGGGUC UCUCUGGUUA GACCA 25

25 (128) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 28
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 127:

CACACAACAG ACGGGCACAC ACUACUUG 28

(129) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:

35 (A) LENGTH: 25
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single

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(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 128:
CUGAGGGAUC UCUAGUUACC AGAGU 25

(130) INFORMATION FOR SEQ ID NO: 129:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 129:
CUCUGGUAAC UAGAGAUCCC UCA 23

(131) INFORMATION FOR SEQ ID NO: 130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
15 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 130:
GUUCGGGCGC CACUGCUAGA GAU 23

20 (132) INFORMATION FOR SEQ ID NO: 131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
25 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 131:
GCAAGCCGAG UCCUGCGUCG AGA 23

(133) INFORMATION FOR SEQ ID NO: 132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
30 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 132:
35 UGCGAGAGCG UCAGUAUUAA GCGG 24

(134) INFORMATION FOR SEQ ID NO: 133:

(i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 36
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

5 (ii) SEQUENCE DESCRIPTION : SEQ ID NO: 133:
CUACUUUGGA AUAUUGCUGG UGAUCCUUUC CAUCCC 36
(135) INFORMATION FOR SEQ ID NO: 134:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 33
10 (B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 134:
CCAAUCCCCC CUUUUCUUUU AAAAUUGUGG AUG 33

15 (136) INFORMATION FOR SEQ ID NO: 135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
20 (D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 135:
CUCGCCACUC CCCAGUCCCG CCA 24
(137) INFORMATION FOR SEQ ID NO: 136:

(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 136:
30 CCGCUUAAUA CUGACGCUCU CGCA 24
(138) INFORMATION FOR SEQ ID NO: 137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 36
(B) TYPE: nucleic acid
35 (C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 137:

GGGAUGGAAA GGAUCACCA?G CAAUAUUGCA AAGUAG 36

(139) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 33
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 138:

10 CAUCCACAAU UUUAAAAGAA AAGGGGGGAU UGG 33

(140) INFORMATION FOR SEQ ID NO: 139:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 24
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) SEQUENCE DESCRIPTION : SEQ ID NO: 139:

UGGGCGGGAC UGGGAGUGG CGAG 24

The claims defining the invention are as follows:

1. A detection probe able to detect the presence of Human Immunodeficiency Virus nucleic acid comprising a sequence able to hybridise to an HIV-1 nucleic acid target region selected from the group consisting of:

- 5 (SEQ ID NO: 3) ATTTGCATGGCTGCTTGATGTCCCCCACT,
 (SEQ ID NO: 5) GTCATCCATCCTATTTGTTTCCTGAAGGGTACTAGTAG,
 (SEQ ID NO: 6) CTCCCTGACATGCTGTCATCATTCTTCTAGTG,
 (SEQ ID NO: 7) GTGGAAGCACATTGTACTGATATCTAATCCC,
 (SEQ ID NO: 8) GCTCCTCTATTTTTGTTCTATGCTGCCCTATTTCTAA,
 10 (SEQ ID NO: 9) CCTTTGTGTGCTGGTACCCATGC,
 (SEQ ID NO: 11) AAAGCCTTAGGCATCTCCTATGGCAGGAA,
 (SEQ ID NO: 13) CAAGGCAAGCTTTATTGAGGCTTAAGCAGTGGG,
 (SEQ ID NO: 14) ATCTCTAGCAGTGGCGCCCGAACAGGGA,
 (SEQ ID NO: 83) AGUGGGGGGACAUCAAGCAGCCAUGCAAU,
 15 (SEQ ID NO: 85) CUACUAGUACCCUUCAGGAACAAAUAGGAUGGAUGAC,
 (SEQ ID NO: 86) CACUAGAAGAAAUGAUGACAGCAUGUCAGGGAG,
 (SEQ ID NO: 87) GGGAUUAGAUUAUCAGUACAAUGUGCUUCCAC,
 (SEQ ID NO: 88) UUAGAAAUAGGGCAGCAUAGAACAAAUAGAGGAGC,
 (SEQ ID NO: 89) GCAUGGGUACCAGCACAAAGG,
 20 (SEQ ID NO: 91) UUCCUGCCAUAAGGAGAUGCCUAAGGCUUU,
 (SEQ ID NO: 93) CCCACUGCUUAAGCCUCAUAAAGCUUGCCUUG, and
 (SEQ ID NO: 94) UCCCUGUUCGGGCGCCACUGCUAGAGAU,

wherein under hybridization assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridise to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human hepatitis virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-target nucleic acid under said conditions.

2. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 3 and SEQ ID NO: 83.

3. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 5 and SEQ ID NO: 85.

4. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 6 and SEQ ID NO: 86.

35 5. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 7 and SEQ ID NO: 87

6. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 8 and SEQ ID NO: 88.

7. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 9 and SEQ ID NO: 89



8. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 11 and SEQ ID NO: 91

9. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 13 and SEQ ID NO: 93.

5 10. The detection probe of claim 1, wherein said HIV-1 target region is selected from the group consisting of: SEQ ID NO: 14 and SEQ ID NO: 94.

11. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 3, SEQ ID NO: 55, SEQ ID NO: 69, and SEQ ID NO: 83.

10 12. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group of SEQ ID NO: 5, SEQ ID NO: 71, SEQ ID NO: 57, and SEQ ID NO: 85.

13. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 6, SEQ ID NO: 58, SEQ ID NO: 72, and SEQ ID NO: 86.

14. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 7, SEQ ID NO: 59, SEQ ID NO: 73, and SEQ ID NO: 87.

15 20 15. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 8, SEQ ID NO: 60, SEQ ID NO: 74, and SEQ ID NO: 88.

16. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 9, SEQ ID NO: 61, SEQ ID NO: 75, and SEQ ID NO: 89.

25 17. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 11, SEQ ID NO: 63, SEQ ID NO: 77, and SEQ ID NO: 91.

30 18. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 13, SEQ ID NO: 65, SEQ ID NO: 79, and SEQ ID NO: 93.

19. The detection probe of claim 1, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 14, SEQ ID NO: 66, SEQ ID NO: 80, and SEQ ID NO: 94.

35 20. The detection probe of any one of claims 11-19, wherein said detection probe consists of said sequence and an optionally present label.

21. A probe mix comprising the detection probe of any one of claims 1-20; an optionally present helper oligonucleotide; and an optionally present amplification oligonucleotide; provided that at least one of said optionally present amplification oligonucleotide and said optionally present helper oligonucleotide is present.



22. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 2 or 11, and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 23 and SEQ ID NO: 103, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 25 and SEQ ID NO: 105.

23. The probe mix of claim 22, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 3, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 23 joined at its 5' end to a sequence recognized by an RNA polymerase; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 25.

24. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 3 or 12; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 26 and SEQ ID NO: 106, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 28 and SEQ ID NO: 108.

25. The probe mix of claim 24, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 5; said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 26 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 26; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 28.

26. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 4 or 13; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 29 and SEQ ID NO: 109, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 30 and SEQ ID NO: 110.

27. The probe mix of claim 26, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 6, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 29 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 29; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 30.

28. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 5 or 14; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 31 and SEQ ID NO: 111, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 32 and SEQ ID NO: 112.

29. The probe mix of claim 28, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 7, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 31 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 31; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 32.



30. The probe mix of claim 29, further comprising the presence of a helper oligonucleotide, said helper oligonucleotide consisting of a sequence selected from the group consisting of: SEQ ID NO: 16 and SEQ ID NO: 133.

31. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 5 or 14; and said probe mix comprises a helper oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 16, SEQ ID NO: 96, SEQ ID NO: 133, and SEQ ID NO: 137.

32. The probe mix of claim 31, wherein said detection probe consists of a detectable label and a sequence selected from the group consisting of: SEQ ID NO: 7 and SEQ ID NO: 73, and said helper oligonucleotide consists of a sequence selected from the group consisting of SEQ ID NO: 16, and SEQ ID NO: 133.

33. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 6 or 15; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 33 and SEQ ID NO: 113, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 34 and SEQ ID NO: 114.

34. The probe mix of claim 33, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 8, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 33 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 33; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 34.

35. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 7 or 16; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 35 and SEQ ID NO: 115, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 36 and SEQ ID NO: 116.

36. The probe mix of claim 35, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 9, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 35 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 35; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 36.

37. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 8 or 17; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 42 and SEQ ID NO: 122, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 43 and SEQ ID NO: 123.

38. The probe mix of claim 37, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 11, said first amplification oligonucleotide consists of the sequence of SEQ ID NO: 43 and a sequence recognized by



an RNA polymerase joined to the 5' end of SEQ ID NO: 43; and said second amplification oligonucleotide consists of the sequence of SEQ ID NO: 42.

39. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 9 or 18; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 46 and SEQ ID NO: 126, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 127, SEQ ID NO: 128 and SEQ ID NO: 130.

40. The probe mix of claim 21, wherein said detection probe is that provided in any one of claims 10 or 19; and said probe mix comprises a first amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 49 and SEQ ID NO: 129, and a second amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 51 and SEQ ID NO: 131.

41. A probe mix comprising: a detection probe able to hybridize to a HIV-1 target region selected from the group consisting of: SEQ ID NO: 1 and SEQ ID NO: 81;

wherein under hybridisation assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe: target duplex and does not hybridize to non-target nucleic acid from Human Immunodeficiency virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human Hepatitis B virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-target nucleic acid under said conditions;

an optionally present helper oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID No: 95, SEQ ID NO: 132, and SEQ ID NO: 136,

and an optionally present amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 99, and SEQ ID NO: 100;

wherein at least one of said optionally present amplification oligonucleotide and said optionally present helper oligonucleotide is present.

42. The probe mix of claim 41, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 1, SEQ ID NO: 53, SEQ ID NO: 67, and SEQ ID NO: 81.

43. The probe mix of claim 42, comprising a helper oligonucleotide consisting of a sequence selected from the group consisting of SEQ ID NO: 15, SEQ ID NO: 95, SEQ ID NO: 132, and SEQ ID NO: 136.

44. The probe mix of claim 43, wherein said detection probe consists of a detectable label and the sequence of either SEQ ID NO: 1 or SEQ ID NO: 67, and said helper oligonucleotide consists of the sequence of either SEQ ID NO: 15 or SEQ ID NO: 132.



45. The probe mix of claim 44, wherein said detection probe consists of said detectable label and the sequence of SEQ ID NO: 1, said helper oligonucleotide consists of the sequence of SEQ ID NO: 15, and said probe mix further comprises a first amplification oligonucleotide consisting of the sequence of SEQ ID NO: 19 and a second
 5 oligonucleotide consists of the sequence of SEQ ID NO: 20 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 20.

46. A probe mix comprising:

a detection probe able to hybridize to a HIV-1 target region selected from the group consisting of: SEQ ID NO: 2 and SEQ ID NO: 82;

10 wherein under hybridization assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe: target duplex and does not hybridize to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human Hepatitis B virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-
 15 target nucleic acid under said conditions; and

an amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 101, and SEQ ID NO: 102.

47. The probe mix of claim 46, wherein said detection probe comprises a
 20 sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 54, SEQ ID NO: 68, and SEQ ID NO: 82.

48. The probe mix claim 46, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 2, and said probe mix comprises a first amplification oligonucleotide consisting of the sequence of SEQ ID NO: 21 and a second
 25 amplification oligonucleotide consisting of the sequence of SEQ ID NO: 22 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 22.

49. A probe mix comprising:

a detection probe able to hybridize to a HIV-1 target region selected from the group consisting of: SEQ ID NO: 4 and SEQ ID NO: 84;

30 wherein under hybridization assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridize to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human Hepatitis B virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-
 35 target nucleic acid under said conditions; and

an amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 24, SEQ ID NO: 27, SEQ ID NO: 104 and SEQ ID NO: 107.

50. The probe mix of claim 49, wherein said detection probe comprises a
 40 sequence selected from the group consisting of: SEQ ID NO: 4, SEQ ID NO: 56, SEQ ID NO: 70, and SEQ ID NO: 84.



51. The probe mix of claim 49, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 4, and said probe mix comprises a first amplification oligonucleotide consisting of the sequence of SEQ ID NO: 27 and a second amplification oligonucleotide consisting of the sequence of SEQ ID NO: 24 and a sequence recognised by an RNA polymerase joined to the 5' end of SEQ ID NO: 24.

52. A probe mix comprising:

a detection probe able to hybridise to a HIV-1 target region selected from the group consisting of: SEQ ID NO: 10 and SEQ ID NO:90;

wherein under hybridization assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex; and does not hybridize to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human Hepatitis B virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-target nucleic acid under said conditions;

an optionally present helper oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 97, SEQ ID NO: 134, and SEQ ID NO: 138;

and an optionally present amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 39, SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 119, SEQ ID NO: 120, and SEQ ID NO: 121;

wherein at least one of said optionally present amplification oligonucleotide and said optionally present helper oligonucleotide is present.

53. The probe mix of claim 52, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 10, SEQ ID NO: 62, SEQ ID NO: 76, and SEQ ID NO: 90.

54. The probe mix of claim 53, comprising a helper oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 17, SEQ ID NO: 97, SEQ ID NO: 134, and SEQ ID NO: 138.

55. The probe mix of claim 54, wherein said detection probe consists of a detectable label and the sequence of either SEQ ID NO: 10 or SEQ ID NO: 76, and said helper oligonucleotide consists of the sequence of either SEQ ID NO: 17 or SEQ ID NO: 134.

56. The probe mix claim 55, wherein said detection probe consists of said detectable label and the sequence of SEQ ID No: 10, said helper oligonucleotide consists of the sequence of SEQ ID NO: 17, and said probe mix further comprises a first amplification oligonucleotide consisting of either the sequence of SEQ ID NO: 40 or SEQ ID NO: 41, and a second amplification oligonucleotide consists of the sequence of SEQ ID NO: 39 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 39

57. A probe mix comprising:



a detection probe able to hybridize to a HIV-1 target region selected from the group consisting of: SEQ ID NO: 12 and SEQ ID NO: 92;

wherein under hybridisation assay conditions said detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridize to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human Hepatitis B virus serotype ADW to form a detectable probe:non-target duplex indicating the presence of said non-target nucleic acid under said conditions;

an optionally present helper oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 18, SEQ ID NO: 98, SEQ ID NO: 135, and SEQ ID NO: 139; and

an optionally present amplification oligonucleotide comprising a sequence selected from the group consisting of: SEQ ID NO: 44, SEQ ID NO: 45, SEQ ID NO: 124, and SEQ ID NO: 125;

wherein at least one of said optionally present amplification oligonucleotide and said optionally present helper oligonucleotide is present.

58. The probe mix of claim 57, wherein said detection probe comprises a sequence selected from the group consisting of: SEQ ID NO: 12, SEQ ID NO: 64, SEQ ID NO: 78, and SEQ ID NO: 92.

59. The probe mix of claim 58, comprising a helper oligonucleotide comprising a sequence selected from the group consisting of SEQ ID NO: 18, SEQ ID NO: 98, SEQ ID NO: 135, and SEQ ID NO: 139.

60. The probe mix of claim 59, wherein said detection probe consists of a detectable label and the sequence of either SEQ ID NO: 12 or SEQ ID NO: 78, and said helper oligonucleotide consists of either SEQ ID NO: 18 or SEQ ID NO: 135.

61. The probe mix claim 60, wherein said detection probe consists of a detectable label and the sequence of SEQ ID NO: 12, said helper oligonucleotide consists of the sequence of SEQ ID NO: 18, and said probe mix further comprises a first amplification oligonucleotide consisting of the sequence of SEQ ID NO: 45 and a second amplification oligonucleotide consists of the sequence of SEQ ID NO: 44 and a sequence recognized by an RNA polymerase joined to the 5' end of SEQ ID NO: 44.

62. A method for determining whether HIV-1 may be present in a sample and distinguishing the presence of HIV-1 from HIV-2 comprising the steps of:

- a) contacting said sample with the detection probe of any one of claims 1-20; and
- b) detecting whether said probe:target duplex has formed using said hybridisation conditions to indicate whether HIV-1 may be present in said sample and to distinguish the presence of HIV-1 from HIV-2.

63. A method for determining whether HIV-1 may be present in a sample and distinguishing the presence of HIV-1 from HIV-2 comprising the steps of:

- a) contacting said sample with the probe mix of any one of claims 21-60;



and b) detecting whether said probe:target duplex has formed using said hybridization conditions to indicate whether HIV-1 may be present in said sample and to distinguish the presence of HIV-1 from HIV-2;

provided that if said probe mix comprises one or more amplification
5 oligonucleotides, HIV-1 nucleic acid if present is amplified using said oligonucleotides prior to said step (b).

64. A method for detecting Human Immunodeficiency Virus type 1 nucleic acid in a sample, wherein under hybridization assay conditions a detection probe hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridise
10 to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human hepatitis virus serotype ADW, substantially as hereinbefore described with reference to any one of the Examples.

65. A method for amplifying Human Immunodeficiency Virus type 1 nucleic acid in a sample, wherein under hybridization assay conditions a detection probe
15 hybridizes to said HIV-1 target region to form a detectable probe:target duplex and does not hybridise to non-target nucleic acid from Human Immunodeficiency Virus type 2, Human T-Cell leukemia virus type 1, Human T-cell virus type 2, and Human hepatitis virus serotype ADW, substantially as hereinbefore described with reference to any one of the Examples.

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