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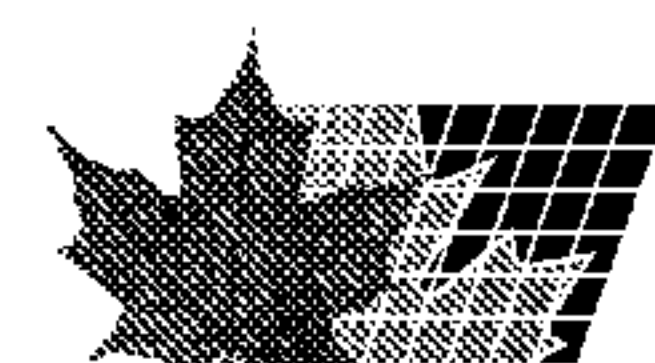
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The present invention relates to the detection of HIV by amplification and PCR-based methods.



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(57) Abstract: The present invention relates to the detection of HIV by amplification and PCR-based methods.

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HIV TYPE AND SUBTYPE DETECTION

5 FIELD OF THE INVENTION

The present invention relates to the field of HIV and HIV subtypes. More precisely, the invention relates to the detection of HIV types and subtypes, and especially to the multiplex detection of HIV types and subtypes.

10 BACKGROUND ART

HIV (Human Immunodeficiency Virus) is the virus responsible for the acquired immunodeficiency syndrome (AIDS), and belongs to the human retrovirus family. AIDS is now considered as one of the greatest threats to human health. An HIV-infected individual can transmit the disease, although
15 remain asymptomatic for years.

The suspected etiological agent responsible for AIDS was independently identified in 1983-1984 by several research groups (see e.g. Barre-Sinoussi et al., *Science* 220:868-871; Montagnier et al., in *Human T-Cell Leukemia Viruses* (Gallo, Essex & Gross, eds.); Vilmer et al., *The Lancet* 1:753), and
20 HIV nomenclature was subsequently unified.

The HIV family comprises several types and subtypes. HIV1 viruses can be classified according to subtypes. Examples of HIV1 sub-types include HIV1-M and HIV1-O. Similarly, HIV2 viruses encompass various sub-types, e.g. HIV2-A and HIV2-B.

25

For drug development assays, prophylaxis, as well as for treatment of AIDS, it has now become of great importance to be able to quickly and easily identify and quantify the group(s), type(s) and subtype(s) of HIV viruses present in a given sample.

30 By HIV group, we herein understand any HIV group, irrespective of it being known at the priority date or not. Various HIV groups are known in the art,

and are described in the corresponding literature and databases, e.g. ncbi on the internet. Examples thereof include HIV1-M and HIV1-O.

By HIV subtype, we herein understand any HIV subtype, irrespective of it being known at the priority date or not. Various HIV subtypes are known in the art, and are described in the corresponding literature and databases, e.g. ncbi on the internet.

By HIV isolate, we herein understand any HIV isolate or strain, irrespective of it being known at the priority date or not. Various HIV isolates are known in the art, and are described in the corresponding literature and databases, e.g. ncbi on the internet. Some isolates are regarded as references. Examples thereof include K03455, L20587 and M30502.

A possible approach could rely on the development of specific antibodies. However, in terms of sensitivity and specificity, a PCR-based approach usually looks very promising. Also, it generally offers the possibility to work on small samples.

Amplification methods, especially polymerase chain reaction (PCR) and PCR-based methods, e.g. reverse-transcriptase PCR (RT-PCR) and PCR are known in the art (*Molecular Cloning: A Laboratory Manual*, Maniatis, Fritsch, and Sambrook, CSHL Press; *Molecular Biology of the Cell*, Alberts et al.; *PCR Primer: A Laboratory Manual*, Dieffenbach and Dveksler, CSHL Press; *The Polymerase Chain Reaction*, Mullis, Ferré, and Gibbs, Birkhauser Boston Press; Gene quantification, Ferré, Birkhauser Boston Press.)

These methods are generally very efficient tools for the qualitative and quantitative analysis of complex biological samples.

However, the efficiency of these techniques typically crucially depends on the design and the choice of primers.

There is prior art describing HIV1-specific primers (US 5 712 385; EP 1 043 407; WO 03/020878; EP 1 344 837). There is also prior art describing HIV2-specific primers (US 5 962 665).

Depending upon the working conditions, at least some of these prior art primers may show a sufficient HIV1 or HIV2 specificity, thereby allowing for

a specific detection of HIV1 and HIV2. However, to the applicant's knowledge, none of them allows for:

- a real-time quantitative specific detection of said subtypes, or for
- a detection of said subtypes in multiplex which would remain specific,
- 5 or for
- a detection of said subtypes in multiplex which would remain quantitative, even when implemented in real-time.

Such a real-time quantitative multiplex specific detection would however be
10 more reliable and informative on the patient's actual infection stage. It would thus give access to a more accurate diagnosis, and as a consequence, would allow to more accurately balance the positive against the deleterious effects of a given treatment. It would allow adjusting or choosing the treatment which should be the most efficient to the particular patient being diagnosed.

15 Such a real-time quantitative multiplex specific detection would also have the advantage of being faster and easier to run, especially on a large scale.

DESCRIPTION OF THE INVENTION

The present invention provides a process for HIV detection.

20 The present invention provides oligonucleotides, including primers and probes, and sets thereof, which are suitable for the detection of HIV.

In this respect, the present invention also relates to the field of amplification, PCR and PCR-based methods, as well as diagnostics.

25 By PCR or PCR reaction, we hereby understand any PCR-based method. This includes standard PCR, qualitative, quantitative and semi-quantitative PCR, real-time PCR, reverse-transcriptase PCR (RT-PCR), simplex and multiplex PCR, and the like.

By real-time PCR, we hereby understand any PCR-based method allowing for
30 monitoring of fluorescence emitted during the reaction as an indicator of

amplicon production during each PCR cycle as opposed to the endpoint detection by conventional PCR methods.

By quantitative PCR, we hereby understand any PCR-based method allowing for the estimation of the initial amount of a given PCR target in a given sample.

By multiplex PCR, we hereby understand any PCR reaction aiming at the amplification of more than one target. For instance, multiplex PCR include duplex PCR (two targets), triplex PCR (three targets), and higher multiplex PCR. Multiplex PCR includes PCR reactions with more than one primer pair, for instance two primer pairs. In this case, there might be four different primers, but it is also possible for the two primer pairs to have one primer in common, e.g. the forward primer, and to have two distinct reverse primers. Multiplex PCR also includes PCR reactions with a unique primer pair, but with more than one probe.

By oligonucleotide, we hereby understand any short polymer of nucleotides, wherein nucleotides can be ribonucleotides, deoxyribonucleotides, dideoxyribonucleotides, degenerated nucleotides, and the like. Said oligonucleotides are preferably single-stranded. The length of said oligonucleotides can vary, and is usually under 150 nucleotides (nt), preferably in the range of 10-100 nt, more preferably 15-60 nt, even more preferably 18-50 nt. Said oligonucleotides can bear chemical modifications, such as tagging or marking, for instance radioactive, fluorescent, biotinylated, dig labelling. An oligonucleotide according to the invention can be either forward (sense) or reverse (antisense). In addition, it should be stressed, that although preferred functions may be mentioned in relation to some oligonucleotides according to the present invention, it is obvious that a given oligonucleotide may assume several functions, and may be used in different ways according to the present invention. For example, an oligonucleotide can be used either as a primer, or as a probe. Also, when an oligonucleotide is described as being useful as an amplicon-targeting probe, the skilled person understands that the complementary sequence of this oligonucleotide is equally useful as a probe to target the same amplicon. Moreover, it is also

obvious, that any primer suitable for a multiplex assay, can also, within the meaning of the present invention, be used in a simplex protocol. The same applies to a primer suitable for a real-time protocol, which can also be used in the framework of an end-point assay within the meaning of the present invention.

Oligonucleotides according to the invention especially include PCR primers and probes. Unless otherwise stated, nucleic acid sequences are given in the 5' to 3' direction. Said oligonucleotides can be under many forms, e.g. under dry state, in solution/suspension with the desired solvent and the desired concentration. The skilled person would know, which solvents, concentrations, storage conditions are suitable for the oligonucleotides of the invention. In particular, the skilled person would know how to prepare said oligonucleotides as stock solutions. The oligonucleotides according to the invention can also assume various degrees of purity, as can be judged by those skilled in the art, e.g. by HPLC chromatography.

By set of oligonucleotides, we hereby understand any combination comprising at least one oligonucleotide, preferably at least two, e.g. 2-10 oligonucleotides. Said set can thus comprise one PCR primer, or a pair of PCR primers, or a probe, or a probe and a pair of primers. Said oligonucleotides can be separately kept, or partially mixed, or entirely mixed.

The notion of primer or PCR primer is known to those skilled in the art. For example, it includes any oligonucleotide able to anneal to a target template under suitable stringency conditions, and allowing for polymerase strand elongation. The typical length of said primer is 15-30 nt, preferably 18, 19, 20, 21, 22, 23, 24 or 25 nt.

The notion of probe is also known to those skilled in the art. For example, it includes any oligonucleotide able to anneal to a target template under the desired hybridization conditions. The typical length of said probe is 20-55 nt, preferably 15-60 nt, more preferably 20-55 nt, more preferably 30-50 nt, more preferably 35-45 nt. Preferably, said probe is fluorescently labelled. However, it is clear to those skilled in the art that under certain conditions, one may use a primer as a probe and vice-versa. Moreover, it is herein stressed that the

products according to the present invention, especially, inter alia, oligonucleotides, are not limited to the intended use herein mentioned, but rather are to be broadly construed, irrespective of the indicated destination. For instance, a claim to a product (oligonucleotide) for a particular use should be
5 construed as meaning a product (oligonucleotide) which is in fact suitable for the stated use. Thus, an oligonucleotide suitable for use as a primer in a multiplex protocol is also clearly adapted to a simplex protocol within the meaning of the present invention.

Various formats (types) of probes, including TaqmanTM probes (hydrolysis
10 probes), molecular beaconsTM (beacon probes or molecular beacon probes), and ScorpionTM probes are known in the art.

In a preferred embodiment, the probes according to the invention can all be synthesized and used in the molecular beacon format.

The structure of molecular beacons is as follows. A short nucleotide sequence
15 (so-called beacon arm) which is unrelated to the target sequence is thus covalently linked to both ends of the probe. A short unrelated arm is thus linked in 5' of the probe, and is labelled with a fluorescent moiety (i.e. fluorescent dye or fluorescent marker). Another but still unrelated arm is linked to the 3' end of probe and is labelled with a fluorescence quenching
20 moiety. Thus, molecular beacons have a fluorophore and a quencher at opposite ends. The 5' short arm is totally complementary to the one in 3' so that they can anneal together, and thus can assume a hairpin structure when unhybridized to the target in solution. In this hairpin conformation, the quencher and the fluorescent dye are close enough to each other to allow
25 efficient quenching of the fluorophore. However, when the probe encounters a target molecule, annealing is favoured with respect to the hairpin conformation when values of beacon arm T_m and probe T_m are suitably chosen (theoretically: probe $T_m >$ beacon arm $T_m >$ primer T_m , wherein T_m is the melting temperature of interest). The fluorophore and quencher move away
30 from each other and the fluorophore can then fluoresce when illuminated by suitable light excitation. As PCR proceeds, amplification product accumulates, and the amount of fluorescence at any given cycle depends on the amount of

amplification product present at that time. (See e.g. Sanjay Tyagi and Fred Russell Kramer, *Nature Biotechnology* 1996, volume 14, pages 303-308; *Nature Biotechnology* 1998, volume 16, pages 49-53).

(Remark: It is also possible to link the fluorophore at the 3' end, while
5 attaching the quencher at the 5' end.)

Schematically, said probe can have the following formulae (molecular beacon format):

5' Fluorophore-(arm1)-probe-(arm2)-Quencher 3'

5' Quencher-(arm1)-probe-(arm2)-Fluorophore 3'

10 wherein arm1 and arm2 can be any short nucleotide sequences, e.g. in the range of 3-10 nucleotides, preferably 5, 6, 7 nucleotides, allowing for the hairpin structure formation under suitable stringency conditions, i.e. arm1 and arm2 are totally complementary to anneal under the desired stringency conditions (standard PCR stringency conditions include, for example, an
15 annealing temperature of 55 to 65°C and an Mg concentration of 4 to 8 mM). However, arm1 and arm2 are unrelated to the target sequence of the probe, i.e. the hairpin conformation resulting from the annealing between arm1 and arm2 is essentially the only possible secondary structure for the probe when unhybridized. The skilled person would know how to choose such arms for a
20 given probe.

For example, possible beacon formats include:

TGCGC-(probe sequence)-GCGCA
GCGCA-(probe sequence)-TGCGC
AGCGC-(probe sequence)-GCGCT
25 GCGCT-(probe sequence)-AGCGC
CGCGA-(probe sequence)-TCGCG
CGCGC-(probe sequence)-GCGCG.

By fluorophore, it is herein understood any fluorescent marker/dye known in the art. Examples of such suitable fluorescent markers include Fam, Hex, Tet,
30 Joe, Rox, Tamra, Max, Edans, Cy dyes such as Cy5, Fluorescein, Coumarin, Eosine, Rhodamine, Bodipy, Alexa, Cascade Blue, Yakima Yellow, Lucifer Yellow and Texas Red (all of them are Trade-Marks).

By quencher, we herein understand any quencher known in the art. Examples of such quenchers include Dabcyl, Dark Quencher, Eclipse Dark Quencher, ElleQuencher, Tamra, BHQ and QSY (all of them are Trade-Marks).

5 The skilled person would know which combinations of dye/quencher are suitable when designing a probe.

In a preferred embodiment according to the invention, spectral properties of said probes can be chosen as to not interfere with each other. In particular, when probes are used in multiplex, each single probe can have its own fluorophore being spectrally significantly different from each other, i.e. the
10 absorption/emission spectra are essentially non-overlapping. This advantageously allows for low-noise multiplex detection for all single probes, making sure that individual signals do not interfere with each other in detection. Examples of dyes which can be used together in multiplex include Fam with Tamra, Fam with Tamra with Texas Red.

15 According to the invention, all the provided oligonucleotides can be either kept separately, or partially mixed, or totally mixed.

Said oligonucleotides can be provided under dry form, or solubilized in a suitable solvent, as judged by the skilled person. Suitable solvents include TE, PCR-grade water, and the like.

20

* * *

Thereafter, sequences are identified by a SEQ ID NO:

The corresponding sequences are given on the tables in the figures appended
25 thereto. In said tables, the standard code for degenerated nucleotides is used. In particular: R is G or A; Y is C or T; W is A or T.

In the given sequences, where several positions are degenerated, it is clear to those skilled in the art that each degenerated position can be chosen independently from each other. For example, RY can be GC, GT, AC, AT,
30 combinations and mixtures thereof. Thus, SEQ ID NO: 52 may be any one of SEQ ID NO: 25 to 51 (see table) or combinations or mixtures thereof, etc.

In addition, in said tables, **d** indicates a degenerated oligonucleotide; **e** denotes an expanded oligonucleotide (i.e. a lengthened version of another oligonucleotide); and **l** designates a loop oligonucleotide.

By loop oligonucleotide, we hereby understand any oligonucleotide, whose 5' end has been modified by addition of a few nucleotides (generally 3, 4, 5, 6 or 7 nt) so as to be complementary to the 3' end of said any oligonucleotide. Thus, said loop oligonucleotide has the advantageous feature of being able to adopt a loop conformation under given stringency conditions. This property is extremely advantageous in increasing the specificity and sensitivity in a PCR protocol, in particular in a multiplex protocol, by avoiding interactions between primers, or between primers and probes.

* * *

The present invention provides a process for the detection of HIV.

In one aspect, the invention provides a process for the detection of at least one HIV target, comprising the step of producing at least one amplicon by means of at least two oligonucleotides,

wherein said oligonucleotides are suitable for use in the specific amplification of at least one reference template sequence selected from the group consisting of:

- positions 4281-4436 (SEQ ID NO: 2) of the K03455 HIV1-M reference isolate; and the fragments of SEQ ID NO: 2 selected from positions 4281-4429 (SEQ ID NO: 306), 4283-4429 (SEQ ID NO: 307), 4283-4431 (SEQ ID NO: 308) of said HIV1-M isolate;
- positions 4336-4491 (SEQ ID NO: 4) of the L20587 HIV1-O reference isolate, and the fragments of SEQ ID NO: 4 selected from positions 4336-4484 (SEQ ID NO: 310), 4338-4484 (SEQ ID NO: 311) of said HIV1-O isolate;
- positions 4889-5036 (SEQ ID NO: 5) of the M30502 HIV2 reference isolate;

- positions 4176-4436 (SEQ ID NO: 1) of the K03455 HIV1-M reference isolate, and the fragment of SEQ ID NO:1 which is identical to positions 4176-4429 (SEQ ID NO: 305) of said HIV1-M isolate; and
- 5 - positions 4231-4491 (SEQ ID NO: 3) of the L20587 HIV1-O reference isolate, and the fragment of SEQ ID NO:3 which is identical to positions 4231-4484 (SEQ ID NO: 309) of said HIV1-O isolate.

The reference template sequences hence correspond to isolated fragments of a
10 determined HIV isolate (i.e. the fragment which is identical to the sequence extending from the indicated positions). The oligonucleotides suitable for use in the specific amplification of at least said reference template sequence are hence selected to target this reference template sequence, in such a location that they would lead to the amplification of this reference template sequence
15 under the form of an isolated fragment.

In another aspect, the invention provides a process for the detection of at least one HIV target, comprising the step of producing at least one amplicon by means of at least two oligonucleotides,

20 wherein said oligonucleotides are 'quantitative friendly' and/or 'multiplex-friendly' primers which are suitable for use in the specific amplification of at least one reference template sequence selected from the group consisting of:

- positions 4281-4436 (SEQ ID NO: 2) of the K03455 HIV1-M reference isolate; and the fragments of SEQ ID NO: 2 selected
25 from positions 4281-4429, 4283-4429, 4283-4431 of said HIV1-M isolate (SEQ ID NO: 306, 307 and 308);
- positions 4336-4491 (SEQ ID NO: 4) of the L20587 HIV1-O reference isolate, and the fragments of SEQ ID NO: 4 selected
30 from positions 4336-4484, 4338-4484 of said HIV1-O isolate (SEQ ID NO: 310 and 311);
- positions 4889-5036 (SEQ ID NO: 5) of the M30502 HIV2 reference isolate;

- positions 4176-4436 (SEQ ID NO: 1) of the K03455 HIV1-M reference isolate, and the fragment of SEQ ID NO:1 which is identical to positions 4176-4429 (SEQ ID NO: 305) of said HIV1-M isolate; and
- 5 - positions 4231-4491 (SEQ ID NO: 3) of the L20587 HIV1-O reference isolate, and the fragment of SEQ ID NO:3 which is identical to positions 4231-4484 (SEQ ID NO: 309) of said HIV1-O isolate.

In another aspect, the invention provides a process for the detection of at least
10 one HIV target, comprising the amplification of at least one reference template sequence selected from the group consisting of:

- positions 4281-4436 (SEQ ID NO: 2) of the K03455 HIV1-M reference isolate; and the fragments of SEQ ID NO: 2 selected from positions 4281-4429, 4283-4429, 4283-4431 of said
15 HIV1-M isolate;
- positions 4336-4491 (SEQ ID NO: 4) of the L20587 HIV1-O reference isolate, and the fragments of SEQ ID NO: 4 selected from positions 4336-4484, 4438-4484 of said HIV1-O isolate;
- positions 4889-5036 (SEQ ID NO: 5) of the M30502 HIV2
20 reference isolate;
- positions 4176-4436 (SEQ ID NO: 1) of the K03455 HIV1-M reference isolate, and the fragment of SEQ ID NO:1 which is identical to positions 4176-4429 of said HIV1-M isolate; and
- 25 - positions 4231-4491 (SEQ ID NO: 3) of the L20587 HIV1-O reference isolate, and the fragment of SEQ ID NO:3 which is identical to positions 4231-4484 of said HIV1-O isolate.

In a preferred embodiment, said process comprises the step of detecting said amplicon by means of at least one probe.

The term amplicon is known to those skilled in the art. By amplicon, we
30 herein understand any amplification product.

Amplification is known in the art, and can be any process involving at least one amplification step, in particular at least one PCR or a PCR-based amplification step.

By 'multiplex-friendly' oligonucleotide, we herein understand any
5 oligonucleotide which can be successfully used in a multiplex (PCR) protocol. In particular, 'multiplex-friendly' oligonucleotides allow specific and sensitive results in a multiplex protocol.

By 'quantitative friendly' oligonucleotide, we herein understand any
10 oligonucleotide which can be successfully used in a quantitative (PCR) protocol. In particular, 'quantitative-friendly' oligonucleotides allow specific, sensitive and quantitative results.

A sequence complementary to another sequence is herein meant as a sequence which is complementary to said other sequence over the entire length of this other sequence.

15

Although the process according to the present invention can advantageously be carried out in the framework of a multiplex protocol, it is also clearly possible to carry it out as a simplex protocol, for example a simplex end-point or qualitative protocol.

20 By reference template sequence, we herein understand any template sequence which can be used as a reference for alignment. For example, some genomes are considered as reference genome. Sequence alignment is known in the art.

Advantageously according to the invention, SEQ ID NO: 1 to 5 and the above-mentioned fragments thereof are reference template sequences sharing the
25 specific technical feature of being suitable references to construct and produce primers which allow for a quantitative detection of at least one of the HIV1-M, HIV1-O, HIV2-A and HIV2-B subtypes. Said reference template sequences are suitable references to construct and produce primers which allow for:

- a real-time quantitative detection of at least one of said HIV
30 subtypes,
- a multiplex detection of at least one of said HIV subtypes,

- a real-time quantitative multiplex detection of at least one of said HIV subtypes.

The reference template sequences of the invention notably comprise the sequences selected from the group consisting of:

- 5 - positions 4176- 4436 (SEQ ID NO: 1) of the K03455 HIV1-M reference isolate, and the fragments of SEQ ID NO:1 selected from positions 4281-4436 (SEQ ID NO: 2), 4281-4429, 4283-4429, 4283-4431, and 4176-4429 of said K03455 HIV1-M reference isolate;
- 10 - positions 4231-4491 (SEQ ID NO: 3) of the L20587 HIV1-O reference isolate, and the fragments of SEQ ID NO:3 selected from positions 4336-4491 (SEQ ID NO: 4), 4336-4484, 4338-4484, and 4231-4484 of said L20587 HIV1-O reference isolate;
- 15 - positions 4889-5036 (SEQ ID NO: 5) of the M30502 HIV2 reference isolate.

Thus, the process according to the invention advantageously allows the specific and sensitive detection of the main HIV groups and/or types and/or subtypes, and of possibly virtually any HIV groups and/or types and/or subtypes, by a real-time quantitative and/or multiplex amplification protocol. Examples of such groups and/or types and/or subtypes covered by the process according to the present invention include HIV1-M subtypes A (A1 and A2), B, C, D, F (F1 and F2), G, H, J and K, but also the recombinant forms AE, AG, AB, DF, BC, CD, BF and BG, and also U (highly divergent). Further examples thereof comprises HIV2 subtypes A, B.

The invention hence provides a HIV detection process by nucleic acid amplification which:

- can be group- and/or type- and/or subtype- specific,
- 30 - is quantitative, and more particularly allows for a real-time quantitative HIV detection,

- can be implemented in multiplex while still remaining specific, and can even be implemented in a (possibly real-time) quantitative multiplex amplification protocol.

5 Thus, the process according to the invention can advantageously facilitate diagnostics procedures by differentiating-HIV1-M and HIV1-O groups from HIV2 group and covering a very broad spectrum of HIV types and/or sub-types using a single procedure.

10 In one aspect of the invention, said reference template sequence is SEQ ID NO: 2, from the K03455 HIV1-M reference isolate, or a fragment of SEQ ID NO: 2 selected from positions 4281-4429, 4283-4429, 4283-4431.

In one embodiment, said reference template sequence is SEQ ID NO: 2, from the K03455 HIV1-M reference isolate, or one of said fragments of SEQ ID
15 NO: 2, and said process is carried out with at least one oligonucleotide selected from the group consisting of:

- SEQ ID NO: 9, 12, 14, 15, 24, 52, 82, 91, 100, 109, 118, 127, 136, and the sequences which are complementary to one of SEQ ID NO: 91, 100, 109, 118, 127, 136 over the entire length
20 of this SEQ ID NO:

In a preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 9, 12, 14, 15, 24, 52, 82.

In another embodiment, said process is carried out with at least one probe
25 selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 118, 127, 136, and the complementary sequences thereof.

In a more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

30 - SEQ ID NO: 9, 12, 14, 15;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 24, 52, 82.

In a still more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 9, 12, 14, 15;

and with at least one primer selected from the group consisting of:

- 5 - SEQ ID NO: 24, 52, 82;

and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 110, 118, 127, 136, and the complementary sequences thereof.

The possible combinations of primers (primer pairs) are thus as follows:

SEQ ID NO:	9	12	14	15
24	X	X	X	X
52	X	X	X	X
82	X	X	X	X

- 10 wherein X indicates that the primers can be combined with each other as a pair, and that each primer pair can in addition be used in combination with any one of the probes selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 110, 118, 127, 136, and the complementary sequences thereof.

15

In another aspect of the invention, said reference template sequence is SEQ ID NO: 4, from the L20587 HIV1-O reference isolate, or a fragment of SEQ ID NO: 4 selected from 4336-4484, 4338-4484.

- 20 In one embodiment of the invention, said reference template sequence is SEQ ID NO: 4, from the L20587 HIV1-O reference isolate or one of said fragments of SEQ ID NO: 4, and said process is carried out with at least one oligonucleotide selected from the group consisting of:

- SEQ ID NO: 148, 157, 167, 170, 179, 189, 190 to 194, and the sequences which are complementary to one of SEQ ID NO: 190 to 194 over the entire length of this SEQ ID NO:
- 25

In a preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 148, 157, 167, 170, 179, 189.

In another embodiment, said process is carried out with at least one probe selected from the group consisting of:

- SEQ ID NO: 190 to 194, and the complementary sequences thereof.

In a more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 148, 157, 167;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 170, 179, 189.

In a still more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 148, 157, 167;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 170, 179, 189;

and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 190 to 194, and the complementary sequences thereof.

The possible combinations of primers (primer pairs) are thus as follows:

SEQ ID NO:	148	157	167
170	X	X	X
179	X	X	X
189	X	X	X

wherein X indicates that the primers can be combined with each other as a pair, and that each primer pair can in addition be used in combination with any one of the probes selected from the group consisting of:

- SEQ ID NO: 190, 191, 192, 193, 194, and the complementary sequences thereof.

In another aspect of the invention, said reference template sequence is SEQ ID NO: 5, from the M30502 HIV2 reference isolate.

In one embodiment of the invention, said reference template sequence is SEQ ID NO: 5, from the M30502 HIV2 reference isolate, and said process is carried out with at least one oligonucleotide selected from the group consisting of:

- 5 - SEQ ID NO: 221, 249, 276, 287, 296, 297 to 303, and the sequences which are complementary to one of SEQ ID NO: 297 to 303 over the entire length of this SEQ ID NO:

In a preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- 10 - SEQ ID NO: 221, 249, 276, 287, 296.

In another embodiment, said process is carried out with at least one probe selected from the group consisting of:

- SEQ ID NO: 297 to 303, and the complementary sequences thereof.

- 15 In a more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 221, 249;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 276, 287, 296.

- 20 In a still more preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 221, 249;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 276, 287, 296;

- 25 and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 297 to 303, and the complementary sequences thereof.

The possible combinations of primers (primer pairs) are thus as follows:

SEQ ID	221	249
NO:		
276	X	X

287	X	X
296	X	X

wherein X indicates that the primers can be combined with each other as a pair, and that each primer pair can in addition be used in combination with any one of the probes selected from the group consisting of:

- SEQ ID NO: 297, 298, 299, 300, 301, 302, 303, and the
5 complementary sequences thereof.

In another aspect of the invention, said reference template sequence is SEQ ID NO: 1, from the K03455 HIV1-M reference isolate, or the fragment of SEQ ID
10 NO:1 which is identical to positions 4176-4429 of said K03455 HIV1-M reference isolate.

In one embodiment of the invention, said reference template sequence is SEQ ID NO: 1, from the K03455 HIV1-M reference isolate, or said fragment thereof, and said process is carried out with at least one oligonucleotide
15 selected from the group consisting of:

- SEQ ID NO: 6, 24, 52, 82, 91, 100, 109, 118, 127, 136, and the sequences which are complementary to one of SEQ ID NO: 91, 100, 109, 118, 127, 136, over the entire length of this SEQ ID NO:

20 In a preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 6, 24, 52, 82.

In another embodiment, said process is carried out with at least one probe selected from the group consisting of:

- 25 - SEQ ID NO: 91, 100, 109, 118, 127, 136, and the complementary sequences thereof.

In a more preferred embodiment, said process is carried out with at least one primer selected which is:

- SEQ ID NO: 6;

30 and with at least one primer selected from the group consisting of:

- SEQ ID NO: 24, 52, 82.

In a still more preferred embodiment, said process is carried out with at least one primer selected which is:

- SEQ ID NO: 6;

5 and with at least one primer selected from the group consisting of:

- SEQ ID NO: 24, 52, 82;

and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 118, 127, 136, and the complementary sequences thereof.

10 The possible combinations of primers (primer pairs) are thus as follows:

SEQ ID	24	52	82
NO:			
6	X	X	X

wherein X indicates that the primers can be combined with each other as a pair, and that each primer pair can in addition be used in combination with any one of the probes selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 118, 127, 136, and the complementary sequences thereof.

15

In another aspect of the invention, said reference template sequence is SEQ ID NO: 3, from the L20587 HIV1-O reference isolate, or the fragment of SEQ ID NO:3 which is identical to positions 4231-4484 of said L20587 HIV1-O reference isolate.

20

In one embodiment of the invention, said reference template sequence is SEQ ID NO: 3, from the L20587 HIV1-O reference isolate, or said fragment thereof, and said process is carried out with at least one oligonucleotide selected from the group consisting of:

- SEQ ID NO: 139, 170, 179, 189, 190 to 194, and the sequences which are complementary to one of SEQ ID NO: 190 to 194 over the entire length of this SEQ ID NO:

25

In a preferred embodiment, said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 139, 170, 179, 189.

In another embodiment, said process is carried out with at least one probe selected from the group consisting of:

- SEQ ID NO: 190 to 194, and the complementary sequences thereof.

In a more preferred embodiment, said process is carried out with at least one primer which is:

- SEQ ID NO: 139;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 170, 179, 189.

In a still more preferred embodiment, said process is carried out with at least one primer which is:

- SEQ ID NO: 139;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 170, 179, 189;

and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 190 to 194, and the complementary sequences thereof.

The possible combinations of primers (primer pairs) are thus as follows:

SEQ ID NO:	170	179	189
139	X	X	X

wherein X indicates that the primers can be combined with each other as a pair, and that each primer pair can in addition be used in combination with any one of the probes selected from the group consisting of:

- SEQ ID NO: 190, 191, 192, 193, 194, and the complementary sequences thereof.

25

In one aspect of the invention, said step of producing at least one amplicon comprises at least one quantitative and/or qualitative, multiplex and/or simplex PCR amplification.

In another aspect of the invention, said step of detecting preferentially includes real-time and/or quantitative and/or end-point detection.

5 In another aspect, the present invention provides an amplicon obtainable by the above-described process, i.e. by implementation of the process of the invention on a HIV-containing sample.

In a further aspect, there is provided an amplification composition comprising at least one amplicon according to the invention. By amplification composition, we herein understand any composition obtainable by
10 amplification, especially by PCR.

The invention is also directed to a polynucleotide suitable for use as a reference template sequence in the design of primers that can be used in multiplex to cover at least HIV1-M, HIV1-O, HIV2-A and HIV2-B in a single
15 amplification run while still offering a real time quantitative amplification thereof. Naturally, the polynucleotides according to the present invention are also suitable for further protocols, including simplex protocols, multiplex protocols, end-point protocols, qualitative protocols, quantitative protocols, combinations thereof, and the like.

20 By polynucleotide, we hereby understand any polymer of nucleotides, wherein nucleotides can be ribonucleotides, deoxyribonucleotides, dideoxyribonucleotides, degenerated nucleotides, and the like. Said nucleotides are preferably single-stranded, but can also be double stranded. The length of said polynucleotides can vary, and is usually under 500
25 nucleotides (nt), preferably in the range of 50-400 nt, more preferably 100-300 nt, even more preferably 150-250 nt.

In a preferred embodiment, said reference template polynucleotide is:

- positions 4281-4436 (SEQ ID NO: 2) of the K03455 HIV1-M reference isolate (see Figure 7), or a fragment of SEQ ID NO: 2
30 selected from 4281-4429, 4283-4429, 4283-4431.

In another embodiment, said reference template polynucleotide is:

- positions 4336-4491 (SEQ ID NO: 4) of the L20587 HIV1-O reference isolate (see Figure 7), or a fragment of SEQ ID NO: 4 selected from 4336-4484, 4338-4484.

In another embodiment, said reference template polynucleotide is:

- 5
- positions 4889-5036 (SEQ ID NO: 5) of the M30502 HIV2 reference isolate (see Figure 7).

In yet another embodiment, said reference template polynucleotide is:

- 10
- positions 4176- 4436 (SEQ ID NO: 1) of the K03455 HIV1-M reference isolate (see Figure 7), or the fragment of SEQ ID NO:1 which is identical to positions 4176-4429 of said K03455 HIV1-M isolate.

In a further embodiment, said reference template polynucleotide is:

- 15
- positions 4231-4491 (SEQ ID NO: 3) of the L20587 HIV1-O reference isolate (see Figure 7), or the fragment of SEQ ID NO:3 which is identical to positions 4231-4484 of said L20587 HIV1-O isolate.

According to the present invention, there is provided an oligonucleotide suitable for HIV detection: see Figures 7 to 21.

- 20
- In one embodiment, the invention provides an oligonucleotide which is selected from the group consisting in:

- SEQ ID NO: 6 to 304, and the sequences which are complementary to one of SEQ ID NO: 83-138, 190-194, 297-304 over the entire length of this SEQ ID NO:

- 25
- In a preferred embodiment, the invention provides an oligonucleotide which is selected from the group consisting in:

- 30
- SEQ ID NO: 6, 9, 12, 14, 15, 24, 52, 82, 91, 100, 109, 118, 127, 136, 139, 148, 157, 167, 170, 179, 189, 190 to 194, 221, 249, 276, 287, 296, 297 to 303, and
 - the sequences which are complementary to one of SEQ ID NO: 91, 100, 109, 118, 127, 136, 190, 191, 192, 193, 194, 297, 298,

299, 300, 301, 302, 303, over the entire length of this SEQ ID NO:

In one aspect, the invention provides an oligonucleotide which is suitable for HIV1-M detection, and which is selected from the group consisting in:

- 5 - SEQ ID NO: 6, 9, 12, 14, 15, 24, 52, 82, 91, 100, 109, 118, 127, 136, and the sequences which are complementary to one of SEQ ID NO: 91, 100, 109, 118, 127, 136 over the entire length of this SEQ ID NO.

In another aspect, the invention provides an oligonucleotide which is suitable
10 for HIV1-O detection and which is selected from the group consisting in:

- SEQ ID NO: 139, 148, 157, 167, 170, 179, 189, 190 to 194, and the sequences which are complementary to one of SEQ ID NO: 190-194, over the entire length of this SEQ ID NO.

In yet another aspect, the invention provides an oligonucleotide which is
15 suitable for HIV2-A detection and which is selected from the group consisting in:

- SEQ ID NO: 221, 249, 276, 287, 296, 297 to 299, and the sequences which are complementary to one of SEQ ID NO: 297-299, over the entire length of this SEQ ID NO.

20

In yet another aspect, the invention provides an oligonucleotide which is suitable for HIV2-B detection and which is selected from the group consisting in:

- 25 - SEQ ID NO: 221, 249, 276, 287, 296, 300 to 303, and the sequences which are complementary to one of SEQ ID NO: 300-303, over the entire length of this SEQ ID NO:

Please note that sequences such as SEQ ID NO: 9 or SEQ ID N:12 are degenerated sequences: SEQ ID NO: 9 with R=A is identical to SEQ ID NO:
30 8; SEQ ID NO: 12 with R=A is identical to SEQ ID NO: 11.

In another aspect, the invention provides the following oligonucleotides, being suitable for the indicated uses:

possible use	SEQ ID NO:
HIV1-M forward primer	6-15
HIV1-M reverse primer	16-82
HIV1-M probe	83-138 (+ complementary sequences thereof)
HIV1-O forward primer	139-167
HIV1-O reverse primer	168-189
HIV1-O probe	190-194 (+ complementary sequences thereof)
HIV2 forward primer	195-249
HIV2 reverse primer	250-296
HIV2-A probe	297-299 (+ complementary sequences thereof)
HIV2-B probe	300-303 (+ complementary sequences thereof)

In a further aspect, the invention provides the following oligonucleotides,
5 being suitable for the indicated uses:

possible use	SEQ ID NO:
HIV1-M forward primer	6, 9, 12, 14, 15
HIV1-M reverse primer	24, 52, 82
HIV1-M probe	91, 100, 109, 118, 127, 136 (+ complementary sequences thereof)
HIV1-O forward primer	139, 148, 157, 167

HIV1-O reverse primer	170, 179, 189
HIV1-O probe	190 to 194 (+ complementary sequences thereof)
HIV2 forward primer	221, 249
HIV2 reverse primer	276, 287, 296
HIV2-A probe	297 to 299 (+ complementary sequences thereof)
HIV2-B probe	300 to 303 (+ complementary sequences thereof)

In a preferred embodiment, the invention provides the following oligonucleotides:

possible use	SEQ ID NO:
HIV1-M loop forward primer	14, 15
HIV1-M loop reverse primer	82
HIV1-M beacon probe	100, 109, 127, 136 (+ complementary sequences thereof)
HIV1-O loop forward primer	167
HIV1-O loop reverse primer	189
HIV1-O beacon probe	191, 192, 194 (+ complementary sequences thereof)
HIV2 loop forward primer	249
HIV2 loop reverse primer	287, 296
HIV2-A beacon probe	298-299 (+ complementary sequences thereof)
HIV2-B beacon probe	301-303

	(+ complementary sequences thereof)
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In a preferred embodiment, any nucleotide according to the invention can be fluorescently labelled.

- 5 In another aspect, the invention provides a set of oligonucleotides suitable for HIV detection.

In one embodiment, the invention provides a set of oligonucleotides suitable for HIV1-M detection.

- 10 In a preferred embodiment, the invention provides a set of oligonucleotides suitable for HIV1-M detection comprising:

- at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 6, 9, 12, 14, 15; and/or
- at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 24, 52, 82; and/or
- 15 - at least one oligonucleotide (probe) selected from the group consisting in SEQ ID NO: 91, 100, 109, 118, 127, 136, and the sequences which are complementary to one of SEQ ID NO: 91, 100, 109, 118, 127, 136, over the entire length of this SEQ ID NO.

- 20 The possible combinations of oligonucleotides are thus as follows:

SEQ ID NO:	6	9	12	14	15
24	X	X	X	X	X
52	X	X	X	X	X
82	X	X	X	X	X

wherein X indicates that the oligonucleotides can be combined with each other as a pair (primer pair), and that each primer pair can in addition be used in combination with any one of the oligonucleotides (probes) selected from the group consisting of:

- SEQ ID NO: 91, 100, 109, 110, 118, 127, 136, and the complementary sequences thereof.

In a further aspect, the invention provides a set of oligonucleotides suitable for
5 HIV1-O detection.

In a preferred embodiment, the invention provides a set of oligonucleotides suitable for HIV1-O detection, comprising:

- at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 139, 148, 157, 167; and/or
- 10 - at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 170, 179, 189; and/or
- at least one oligonucleotide (probe) selected from the group consisting in SEQ ID NO: 190 to 194, and the sequences which are complementary to one of SEQ ID NO: 190-194, over the
15 entire length of this SEQ ID NO.

The possible combinations of oligonucleotides are thus as follows:

SEQ ID NO:	170	179	189
139	X	X	X
148	X	X	X
157	X	X	X
167	X	X	X

wherein X indicates that the oligonucleotides can be combined with each other as a pair (primer pair), and that each primer pair can in addition be used in combination with any one of the oligonucleotides (probes) selected from the
20 group consisting of:

- SEQ ID NO: 190, 191, 192, 193, 194, and the complementary sequences thereof.

In a further aspect, the invention provides a set of oligonucleotides suitable for
25 HIV2-A detection.

In a preferred embodiment, there is provided a set of oligonucleotides suitable for HIV2-A detection, comprising:

- at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 221, 249; and/or
- 5 - at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 276, 287, 296; and/or
- at least one oligonucleotide (probe) selected from the group consisting in SEQ ID NO: 297 to 299 and the sequences which are complementary to one of SEQ ID NO: 297-299 other the
- 10 entire length of this SEQ ID NO.

The possible combinations of oligonucleotides are thus as follows:

SEQ ID NO:	221	249
276	X	X
287	X	X
296	X	X

wherein X indicates that the oligonucleotides can be combined with each other as a pair (primer pair), and that each primer pair can in addition be used in combination with any one of the oligonucleotides (probes) selected from the

15 group consisting of:

- SEQ ID NO: 297, 298, 299 and the complementary sequence thereof.

In a further aspect, the invention provides a set of oligonucleotides suitable for

20 HIV2-B detection.

In a preferred embodiment, there is provided a set of oligonucleotides suitable for HIV2-B detection, comprising:

- at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 221, 249; and/or
- 25 - at least one oligonucleotide (primer) selected from the group consisting in SEQ ID NO: 276, 287, 296; and/or

- at least one oligonucleotide (probe) selected from the group consisting in SEQ ID NO: 300 to 303, and the sequences which are complementary to one of SEQ ID NO: 300-303, over the entire length of this SEQ ID NO.

5 The possible combinations of oligonucleotides are thus as follows:

SEQ ID NO:	221	249
276	X	X
287	X	X
296	X	X

wherein X indicates that the oligonucleotides can be combined with each other as a pair (primer pair), and that each primer pair can in addition be used in combination with any one of the oligonucleotides (probes) selected from the group consisting of:

- 10 - SEQ ID NO: 300, 301, 302, 303, and the complementary sequences thereof.

In a further aspect, the invention provides a set of oligonucleotides suitable for HIV multiplex detection.

15 In a preferred embodiment, there is provided a set of oligonucleotides suitable for HIV detection, preferably multiplex detection, comprising:

- at least one oligonucleotide selected from the group consisting in SEQ ID NO: 91, 100, 109, 118, 127, 136, and the complementary sequences thereof; and/or
- 20 - at least one oligonucleotide selected from the group consisting in SEQ ID NO: 190 to 194, and the complementary sequences thereof; and/or
- at least one oligonucleotide selected from the group consisting in SEQ ID NO: 297 to 299, and the complementary sequences thereof; and/or
- 25

- at least one oligonucleotide selected from the group consisting in SEQ ID NO: 300 to 303, and the complementary sequences thereof; and/or
- combinations thereof.

5 Such a set of oligonucleotides according to the invention may thus comprise any combination thereof of 2, 3, 4, 5 and more of said oligonucleotides.

In a preferred embodiment, such a set of oligonucleotides can be a combination of probes, e.g. a combination of an HIV1-M probe and an HIV1-O probe:

SEQ ID NO:	91	100	109	118	127	136
190	X	X	X	X	X	X
191	X	X	X	X	X	X
192	X	X	X	X	X	X
193	X	X	X	X	X	X
194	X	X	X	X	X	X

10 (X=possible combination, optionally with any further probe(s), eg HIV2-A or HIV2-B probes)

In another embodiment, such a set of oligonucleotides can be a combination of probes, e.g. a combination of an HIV2-Aprobe and an HIV2-B probe:

SEQ ID NO:	297	298	299
300	X	X	X
301	X	X	X
302	X	X	X
303	X	X	X

15 (X= possible combination, optionally with any further probe(s), eg HIV1-M or HIV1-O probes).

Further possibilities include:

SEQ ID NO:	91	100	109	118	127	136
297	X	X	X	X	X	X
298	X	X	X	X	X	X
299	X	X	X	X	X	X
300	X	X	X	X	X	X
301	X	X	X	X	X	X
302	X	X	X	X	X	X
303	X	X	X	X	X	X

(X= possible combination, optionally with any further probe(s), eg for HIV1-O)

and

SEQ ID NO:	190	191	192	193	194
297	X	X	X	X	X
298	X	X	X	X	X
299	X	X	X	X	X
300	X	X	X	X	X
301	X	X	X	X	X
302	X	X	X	X	X
303	X	X	X	X	X

(X= possible combination, optionally with any further probe(s), eg for HIV1-

5 M)

In a preferred embodiment, in any set of oligonucleotides according to the invention, at least one of said oligonucleotides is fluorescently labelled.

- 10 There is further provided an amplicon obtainable by means of at least one oligonucleotide according to the invention, and/or at least one set of nucleotides according to the invention.

There is more particularly provided an amplicon obtainable by amplification from a HIV-containing sample with a pair of primers selected from:

- a primer of SEQ ID NO: 9, 12, 14 or 15, and a primer of SEQ ID NO: 24, 52 or 82; or
- 5 - a primer of SEQ ID NO: 148, 157 or 167, and a primer of SEQ ID NO: 170, 179 or 189; or
- a primer of SEQ ID NO: 221 or 249, and a primer of SEQ ID NO: 276, 287 or 296; or
- 10 - a primer of SEQ ID NO: 6, and a primer of SEQ ID NO: 24, 52 or 82; or
- a primer of SEQ ID NO: 139, and a primer of SEQ ID NO: 170, 179 or 189.

There is also provided an amplicon which has a nucleotide length identical to the nucleotide length of one of the reference template sequences of the invention (i.e. SEQ ID NO: 2, 4, 5, 1 or 3 and the above-mentioned fragments thereof), and which comprises a sequence having a percentage of nucleotide identity of at least 90%, preferably of at least 91%, most preferably of at least 92%, most preferably of at least 93%, most preferably of at least 94%, most preferably of at least 95%, most preferably of at least 96%, most preferably of at least 97%, most preferably of at least 98%, most preferably of at least 99%, most preferably of 100%, with a probe sequence, over the entire length of this probe sequence, wherein this probe sequence is one of the following: SEQ ID NO: 91, 100, 109, 118, 127, 136, 190, 191, 192, 193, 194, 297, 298, 299, 300, 301, 302, 303, and the sequences which are complementary to these SEQ ID NO: over the entire length of these SEQ ID NO.

An amplification composition comprising such an amplicon is also encompassed by the present invention.

It is another object of the present invention to provide with a kit.

In a preferred embodiment, said kit comprises at least one oligonucleotide (primer or probe) according to the invention, as described above.

In another embodiment, said kit comprises at least one primer pair according to the invention, as described above.

In yet another embodiment, said kit comprises at least a set of oligonucleotides according to the invention, for example at least one plurality of probes according to the invention, as described above.

In the kit according to the invention, the oligonucleotides (primers, probes)
5 can be either kept separately, or partially mixed, or totally mixed.

Said oligonucleotides can be provided under dry form, or solubilized in a suitable solvent, as judged by the skilled person. Suitable solvents include TE, PCR-grade water, and the like.

In a preferred embodiment, the kit according to the invention can also contain
10 further reagents suitable for a PCR step, possibly including reagents suitable for an RT-PCR step.

Such reagents are known to those skilled in the art, and include water, like nuclease-free water, RNase free water, DNase-free water, PCR-grade water; salts, like magnesium, potassium; buffers such as Tris; enzymes, including
15 polymerases, such as Taq, Vent, Pfu (all of them Trade-Marks), activable polymerase, reverse transcriptase, and the like; nucleotides like deoxynucleotides, dideoxynucleotides, dNTPs, dATP, dTTP, dCTP, dGTP, dUTP; other reagents, like DTT and/or RNase inhibitors; and polynucleotides like polyT, polydT, and other oligonucleotides, e.g. primers.

20 In another preferred embodiment, the kit according to the invention comprises PCR controls. Such controls are known in the art, and include qualitative controls, positive controls, negative controls, internal controls, quantitative controls, internal quantitative controls, as well as calibration ranges. The internal control for said PCR step can be a template which is unrelated to the
25 target template in the PCR step. Such controls also may comprise control primers and/or control probes. For example, in the case of HIV detection, it is possible to use as an internal control, a polynucleotide chosen within a gene whose presence is excluded in a sample originating from a human body (for example, from a plant gene), and whose size and GC content is equivalent to
30 those from the target sequence.

In a preferred embodiment, the kit according to the invention contains means for extracting and/or purifying nucleic acid from a biological sample, e.g. from blood, serum, plasma. Such means are well known to those skilled in the art.

In a preferred embodiment, the kit according to the invention contains
5 instructions for the use thereof. Said instructions can advantageously be a leaflet, a card, or the like. Said instructions can also be present under two forms: a detailed one, gathering exhaustive information about the kit and the use thereof, possibly also including literature data; and a quick-guide form or a memo, e.g. in the shape of a card, gathering the essential information needed
10 for the use thereof.

In a preferred embodiment, said kit is a diagnostics kit, especially an in vitro diagnostics kit, i.e. an HIV diagnostics kit.

The present invention also relates to the field of diagnostics.

15 The oligonucleotides according to the present invention, and as described above, can be used for the in vitro diagnostics of HIV types and subtypes. In particular, the primers and probes according to the invention can be used for in vitro typing, sub-typing, and quantification of HIV nucleic acids present in an in vitro sample, for instance, in a patient's blood, plasma and/or serum, or in a
20 cell culture supernatant.

It is also an object of the present invention to provide with a method to detect HIV nucleic acid presence in a sample.

In one embodiment, said method comprises the step of providing with at least
25 one sample suspected of comprising at least one target template from at least one HIV type and/or sub-type and/or isolate.

By nucleic acid, we hereby understand any nucleic acid: it can be synthetic or not, recombinant or naturally occurring, linear or circular. This includes DNA and RNA. The nucleic acid can be either single stranded or double stranded or
30 even triple stranded. It can stem from various biological sources, such as micro organisms (bacteria, yeasts, and the like), or higher organisms, like mammal cells. Said nucleic acid can also be of viral nature, e.g. retroviral

nature, like HIV's. The nucleic acid can also comprise total DNA, total RNA, genomic DNA, mitochondrial DNA, plasmidic DNA, BAC DNA, and mixtures thereof. Moreover, the nucleic acid can assume various states of purity.

5 By sample, we hereby understand any kind of sample, naturally occurring or not. Preferably, said sample is from biological origin. Said sample may also stem from a cell culture supernatant. In a preferred embodiment, said sample derives from blood. More preferably, said nucleic acid containing sample derives from serum and/or plasma. Said sample might also result from a
10 preliminary step. For instance, said sample might be obtainable via a purification and/or extraction procedure, e.g. from a blood sample. In particular, said sample may result from a separation and/or purification and/or extraction process carried out on a biological sample. Said sample can also be a control sample. Control samples include samples as qualitative control,
15 positive control, negative control, quantitative control, and calibrating control. Said control can be internal as well as external. Any sample according to the present invention can be present several times. For instance, said sample can be provided as duplicate, as triplicate, as quadruplicate ... as multiplicate, which is advantageous in the case of quantitative experiments.

20 The skilled person is familiar with the notion of target template. Said target template can be any nucleic acid, whose presence is to be assessed in said method. Said target template is possibly, but not necessarily, the nucleic acid to be amplified in said method (PCR amplicon).

Said method may comprise the step of providing with at least one nucleic acid-
25 containing sample suspected of comprising at least one target template from at least one HIV type and/or sub-type.

In one aspect of the invention, said method comprises the step of providing with at least one oligonucleotide according to the invention (e.g. PCR primer and/or probe) and/or at least one set of nucleotides (e.g. a primer pair)
30 according to the invention.

In one embodiment, said method comprises the step of contacting said nucleic acid-containing sample with at least one oligonucleotide according to the

invention and/or at least one set of nucleotides according to the invention, under conditions enabling the annealing of said primer and/or said primer pair and/or said probe onto said template.

In another aspect, said method comprises the step of observing or detecting the
5 presence of an annealed product, thereby revealing the initial presence of an HIV nucleic acid in said sample.

It is a further object of the invention to provide with a method for the quantitative specific detection of HIV types and sub-types in a sample,
10 preferably through real-time quantitative multiplex PCR analysis.

In one embodiment, said method comprises the step of providing with at least one sample suspected of comprising at least one target template from at least one HIV type and/or sub-type.

In another preferred embodiment, said method comprises the step of providing
15 with at least one oligonucleotide according to the invention (e.g. PCR primer and/or probe) and/or at least one set of nucleotides according to the invention (e.g. primer pair).

In a preferred embodiment, said probe is suitable for the detection of a putative HIV amplicon obtainable with said primer pair.

20 In a preferred embodiment according to the invention, spectral properties of said probes can be chosen as to not interfere with each other. In particular, when probes are used in multiplex, each single probe can have its own fluorophore being spectrally significantly different from each other, i.e. the absorption/emission spectra are essentially non-overlapping. This
25 advantageously allows for low-noise multiplex detection for all single probes, making sure that individual signals do not interfere with each other in detection. Examples of dyes which can be used together in multiplex include Fam with Tamra, Fam with Tamra and Texas Red.

In another preferred embodiment, said method comprises the step of
30 contacting said sample, in presence of said oligonucleotide(s) and/or primer(s) and/or pair(s) or primers and/or probe(s) and/or set(s) of oligonucleotides and possibly in the presence of suitable reagents, to the conditions suitable for the

PCR amplification of said target template with said primer pair(s) and/or set(s).

Said PCR amplification can be any PCR reaction, including RT-PCR.

Such suitable reagents are known in the art, and examples thereof include
5 water, like nuclease-free water, RNase free water, DNase-free water, PCR-grade water; salts, like magnesium, potassium; buffers such as Tris; enzymes, including polymerases, such as Taq, Vent, Pfu (all of them Trade-Marks), activable polymerase, reverse transcriptase, and the like; nucleotides like deoxynucleotides, dideoxynucleotides, dNTPs, dATP, dTTP, dUTP, dCTP,
10 dGTP; other reagents, like DTT and/or RNase inhibitors; and polynucleotides like polyT, polydT. Advantageously according to the invention, at least part of these reagents can be used as a pre-mix. The amounts thereof to be used are known to those skilled in the art.

In a preferred embodiment, the primers according to the invention are used in
15 a final concentration range 100-2000nM. Typically, said primers can be used at a final concentration range 200-1500nM, preferably 250-1000nM, more preferably 500-1000nM, even more preferably 600-1000nM.

Probe concentration in a PCR reaction can be optimized, typically by varying the final concentration from 50nM to 1000nM. In a preferred embodiment, the
20 probes according to the invention are used at a final concentration range 50-1000nM, preferably 100-800nM, more preferably 100-600nM, even more preferably 200-600nM.

Said conditions are known to those skilled in the art. They include temperature conditions, in particular thermal cycling conditions, e.g. temperature, duration,
25 number, heating rate of the cycles. In a preferred embodiment, said temperature conditions include conditions suitable for an RT-PCR. In another preferred embodiment, said conditions include conditions suitable for a QPCR. In yet another preferred embodiment, said conditions include conditions suitable for a quantitative RT-PCR.

30 In another embodiment, said method comprises the step of bringing said sample, in the presence of said probe(s) under conditions suitable for the annealing of said probe to said putative amplicon.

In yet another preferred embodiment, said method comprises the step of detecting at least once, preferably real-time, potential amplification products, i.e. whether said probe(s) meet(s) said amplicon to anneal with, preferably for each sample. This advantageously allows for the assessment of the presence of
5 said HIV type and/or sub-type. This can be advantageously achieved by fluorescence intensity measurements. Fluorescence measurement procedures are known in the art. Briefly, the sample is illuminated at around the excitation wavelength of the fluorophore, and emission intensity is measured.

In another embodiment, said method comprises the step of measuring at least
10 once, preferably real-time, the amount of said probe annealed to said amplicon. This can be advantageously achieved by fluorescence intensity measurements. Fluorescence measurement procedures are known in the art. Briefly, the sample is illuminated at around the excitation wavelength of the fluorophore, and emission intensity is measured

15 In another preferred embodiment, said method comprises the step of estimating at least once the number of target template copies initially present in said sample. The skilled person would know how to carry out such a step. For example, this can be advantageously performed having used calibration standards and/or internal controls. Preferably, this step includes the
20 determination of the so-called threshold cycle (CT) for each sample, which correlates to the number of target template copies initially present in said sample.

In a preferred embodiment, at least one step, preferably several steps, more preferably most of the steps of said method can be carried out in a PCR plate.
25 Suitable such PCR plates are known in the art. They include 24-well plates, 48-well plates, 96-well-plates, and 284-well plates. This advantageously ensures that samples can be processed in parallel in said steps. In addition, this allows for high throughput screening, which advantageously saves time.

In another preferred embodiment, at least one step, preferably several steps,
30 more preferably most steps of said method can be carried out in a thermal cycler. Such thermal cycler might be equipped for real-time fluorescence

intensity measurements, in which case said plates can advantageously be of optical-grade.

This application also relates to the amplification of HIV nucleic acids with at least one oligonucleotide and/or PCR primer and/or at least one PCR primer pair and/or at least one probe and/or at least one set of nucleotides according to the invention.

The skilled person can appreciate that the present invention can incorporate any number of the preferred features described above.

Although being suitable for detection via multiplex and/or real-time protocols, the methods, processes, polynucleotides, oligonucleotides, sets of oligonucleotides, amplicons, and kits of the present invention are obviously also suitable for simplex protocols, qualitative protocols, quantitative protocols, end-point detection protocols, and combinations thereof.

The advantages of the products, processes and methods according to the invention will become apparent from the following examples, which are given below as mere illustrations, and are non limitative.

Figures 1A, 1B, 2A, 2B and 3 illustrate HIV2-A and HIV2-B detection according to the present invention (see example 1 below).

Figures 4A and 4B illustrate HIV1-M and HIV1-O detection according to the present invention (see example 2 below).

Figures 5A and 5B illustrate HIV1/HIV2 multiplex detection according to the present invention (see example 4 below).

Figures 6A, 6B and 6C illustrate an HIV2/internal control assay according to the present invention (see example 5 below).

Figures 7 to 21 illustrate sequences according to the present invention.

5 EXAMPLES

Example 1: HIV2 detection (sub-types A and B) (Figures 1A, 1B, 2A, 2B and 3)

10 This example illustrates that the HIV2 primers and probes of the invention allow for RT-PCR specific real-time detection of HIV2 sub-types A and B.

This example involves the use of one pair of HIV2 primers, and two different HIV2 probes. One probe is an HIV2-A probe (sub-type A probe), and the other one is an HIV2-B probe (sub-type B probe). These primers and probes
15 target a 147 bp sequence in HIV2 isolates.

They have the following sequences:

HIV2 forward primer (H2A3f, SEQ ID NO: 195):

AGGAAGRCARACAGCACTCTTC with R=A/G (T_m = 66°C)

HIV2 reverse primer (H2A3r, SEQ ID NO: 250):

20 GGTACTCCRAAGGGGTTTGTCTAT with R=A/G (T_m = 65°)

As already mentioned, R corresponds to an A or G, so that there are 4 forms for the forward primer (AA, AG, GA and GG) et 2 forms for the reverse primer (AG or GA).

HIV2-A probe (HIV2-A probe, SEQ ID NO: 297):

25 GGCCAATAACACACTTGACACACA

HIV2-B probe (HIV2-B probe, SEQ ID NO: 300):

GCCTATCACACACCTGCACACA

Each of these probes is used as a molecular beacon in this example. The target-unrelated beacon arms which have been added at each end of each probe
30 are shown underlined (FAM = fluorophore; DQ = Dark Quencher).

HIV2-A probe in molecular beacon format (HIV2-A beacon probe, SH2A9a, SEQ ID NO: 298):

FAM - AGCGCGGCCAATAACACACTTGCACACAGCGCT – DQ

HIV2-B probe in molecular beacon format (HIV2-B beacon probe, SH2A1b, SEQ ID NO: 301):

FAM - AGCGCGCCTATCACACACCTGCACACAGCGCT – DQ

- 5 Three series of real-time quantitative amplification experiments are performed on a panel of HIV2-positive plasma samples (HIV2-A positive HIV2-B negative samples and HIV2-B positive HIV2-A negative samples):

- a) HIV2 primer pair + HIV2-A beacon probe (H2A3f, H2A3r, SH2A9a, SEQ ID NO: 195, 250, 298),
- 10 b) HIV2 primer pair + HIV2-B beacon probe (H2A3f, H2A3r, SH2A1b, SEQ ID NO: 195, 250, 301),
- c) HIV2 primer pair + HIV2-A beacon probe + HIV2-B beacon probe (H2A3f, H2A3r, SH2A9a, SH2A1b, SEQ ID NO: 195, 250, 298, 301).

Details of the procedure are as follows:

- 15 - Panel of HIV2-positive samples: HIV2-A positive HIV2-B negative plasma samples (sub-type A samples) and HIV2-B positive HIV2-A negative plasma samples (sub-type B samples) are obtained from infected patients (Centre Hospitalier Bichat, Laboratory of Virology, Assistance Publique – Hôpitaux de Paris, France);
- 20 - Negative controls: HIV2-negative plasma;
- Nucleic acid extraction: Kit Qiagen QiaAmp Viral RNA (reference 52904) used in accordance with the manufacturer's recommendations (volume of sample for extraction = 140 microliters);
- Primer pair: 1 microM each per reaction (SEQ ID NO: 11, 12);
- 25 - Probes: FAM-Dark Quencher beacon probes as described above (SEQ ID NO: 298, 301); 0.2 microM each per reaction;
- RT-PCR reagents: RNase-free water, and Qiagen QuantiTect Probe RT-PCR kit (Qiagen reference: 204443) used according to the manufacturer's instructions, [MgCl₂] = 4 mM;
- 30 - Apparatus: IQ-Cycler Bio-Rad;
- Thermal cycling:
30' at 42°C (RT step),

15' at 95°C (polymerase activation step),
 (30'' at 94°C – 30'' at 55°C – 30'' at 72°C) x 50,
 +4°C.

The panel of sub-type A samples and sub-type B samples are submitted to
 5 nucleic acid extraction and RT-PCR amplification using the HIV2 primer pair
 together with either only one of the probes (experiments **a)** and **b)**), or both
 probes (experiment **c)**).

Interpretation of the results: for each assay, one determines a threshold cycle
 (Ct) which is the level of fluorescence that is considered to be significantly
 10 above the background level of fluorescence measured in the early cycles of the
 amplification. The Ct value is inversely proportional to the concentration of
 target: the lower the Ct, the higher the concentration of target.

In the following tables, CT= Threshold Cycle; RFU max = maximal Relative
 Fluorescence Units observed at the end of the PCR run; CTL - = negative
 15 control; N/A= sample whose level of fluorescence is below the background
 level.

Table 1: Results obtained with HIV2 primer pair + HIV2-A molecular beacon
 probe in experiment **a)** (H2A3f, H2A3r, SH2A9a, SEQ ID NO: 195, 250,
 298).

Sub-type A Samples dilution	A1 1/10	CT	20.2
		RFUmax	916
	GS 1/800	CT	22
		RFUmax	1078
	A3 1/10	CT	21.2
		RFUmax	1382
Sub-type B Samples dilution	A4 1/10	CT	22.4
		RFUmax	1221
	B1 1/10	CT	42.9
		RFUmax	67
	B2 1/10	CT	30.9
		RFUmax	265
	B3 1/10	CT	N/A
		RFUmax	50
Negative control	CTL -	CT	N/A
		RFU max	N/A

These results are illustrated by Figures 1A and 1B.

Advantageously according to the invention, the HIV2-A probe (SH2A9a, SEQ ID NO: 298) allows for targeted detection of sub-type A positive samples in combination with the HIV2 primers according to the invention (H2A3f, H2A3r, SEQ ID NO: 195, 250), without cross-detection of sub-type B positive samples (see Table 1, and Figures 1A and 1B).

Table 2: Results obtained with HIV2 primer pair + HIV2-B probe in experiment b) (H2A3f, H2A3r, SH2A1b, SEQ ID NO: 195, 250, 301).

Sub-type A Samples dilution	A1 1/10	CT	35.1	39.5
		RFUmax	84	53
	GS 1/800	CT	36.8	40.5
		RFUmax	81	57
	A3 1/10	CT	26.9	26.7
		RFUmax	159	137
Sub-type B samples	A4 1/10	CT	44	N/A
		RFUmax	55	-
	B1 1/10	CT	21.4	21.5
		RFUmax	1018	996
	B2 1/10	CT	29.6	29.9
		RFUmax	286	292
Negative control	B3 1/10	CT	26.3	27.2
		RFUmax	768	690
	B4 1/10	CT	27	27.1
		RFUmax	832	779
	CTL -	CT	N/A	N/A
		RFU max	N/A	N/A

These results are illustrated by Figures 2A and 2B.

Advantageously according to the invention, the HIV2-B probe (SH2A1b, SEQ ID NO: 301) allows for targeted detection of sub-type B positive samples in combination with the HIV2 primers (H2A3r, H2A3f, SEQ ID NO: 195, 250) of the invention, without cross-detection of sub-type A positive samples (see Table 2, and Figures 2A and 2B).

Table 3: Results obtained with HIV2 primer pair + HIV2-A probe + HIV2-B probe in experiment c) (H2A3f, H2A3r, SH2A9a, SH2A1b, SEQ ID NO: 195, 250, 298, 301).

Sub-type A Samples dilution	A1 1/10	CT	20.3	20.6
		RFU max	1673	1550
	GS 1/800	CT	23.7	23.9
		RFU max	1709	1580
	A3 1/10	CT	21.4	21.5
		RFU max	2439	2201
	A4 1/10	CT	22.6	22.8
		RFU max	1974	1911
Sub-type B samples	B1 1/10	CT	23.3	23.6
		RFU max	1197	1122
	B2 1/10	CT	30.2	30.5
		RFU max	635	672
	B3 1/10	CT	28.6	28.7
		RFU max	814	834
Negative control	CTL -	CT	N/A	N/A
		RFU max	N/A	N/A

5

These results are illustrated by Figure 3.

As a remarkable feature of the invention, the HIV2-A probe (SH2A9a, SEQ ID NO: 298) and the HIV2-B probe (SH2A1b, SEQ ID NO: 301) can be used simultaneously (see Table 3 and Figure 3) and allow for detection of both sub-
types in a one-step multiplex (here duplex) procedure.

As a surprising feature, a synergistic effect is observed for the simultaneous use of both A and B probes (sub-type A probe and sub-type B probe) (duplex protocol), compared to the use of a single probe alone (sub-type A probe or sub-type B probe). The detection signal obtained with a single probe is slightly
less accurate than the respectively obtained with both probes, as can be judged from the comparison of the results obtained in experiment a) with those obtained in experiment c) on sub-type A samples, and from the comparison of the results obtained in experiment b) with those obtained on sub-type B samples in experiment c).

The sets of oligonucleotides (primers and probes) according to the invention thus prove an increased specificity when probes are used simultaneously (multiplex, duplex protocol), as a surprising and unexpected synergistic effect

5

Example 2: HIV1-M / HIV1-O primers and probes (Figures 4A and 4B)

In one run, representative reference panels of HIV1-M positive HIV1-O negative serum samples (HIV1-M panel samples) and representative reference panels of HIV1-O positive HIV1-M negative serum samples (HIV1-O panel samples) are subjected to real-time quantitative RT-PCR amplification with the HIV1-M primers (H1B4f, H1B10r, SEQ ID NO: 7, 16) and an HIV1-M probe (SH1BM5, SEQ ID NO: 119) of the invention.

15 In another run, the same panels are subjected to real-time quantitative RT-PCR amplification with the HIV1-O primers (H1B5f, H1B13r, SEQ ID NO: 140, 168) and a HIV1-O probe (SH1BO2 SEQ ID NO: 191 or SH1B010 SEQ ID N° 194) of the invention

20 The HIV1 primers and probes have the following sequences:

HIV1-M primers:

Forward: TTGGAGAGCAATGGCTAGTGA (H1B4f, SEQ ID NO: 7)

Reverse: TGTGTGCAATCTAGTTGCCATA (H1B10r, SEQ ID NO: 16)

25 HIV1-M probe:

ATAGTAGCCAGCTGTGATAAATGTC (SEQ ID NO: 110)

HIV1-O primers:

Forward: TTGGAGAGCACTAGCTAGTGA (H1B5f, SEQ ID NO: 140)

Reverse: TGTGTGCAATCTATTTGCCATA (H1B13r, SEQ ID NO: 168)

30

HIV1-O probe:

GAAATCATTTGCTAGTTGTCCTAAATGTCATAT (SEQ ID NO: 190)

According to the invention, each probe is produced here as a molecular beacon, with “TGCGC” as target-unrelated arm in 5’, and with “GCGCA” as target-unrelated arm in 3’ (both arms are underlined). The 5’ arm is labelled with a FAM fluorophore, and the 3’ arm with a Dark Quencher.

5

Probe sequences are then the following:

HIV1-M probe in a molecular beacon format:

FAM-TGCGCATAGTAGCCAGCTGTGATAAATGTCGCGCA-DQ
(SH1BM5, SEQ ID NO: 119)

10

HIV1-O probe in a molecular beacon format:

FAM-TGCGCGAAATCATTGCTAGTTGTCCTAAATGTCATATGCGCA-DQ
(SH1BO2, SEQ ID NO: 191)

Details of the RT-PCR procedure are as follows:

- 15 - Panels: BBI Performance Panel reference PRD301 (HIV1-O cultures diluted in human plasma at about 10^5 copies per mL; sample numbers have the format 301-xx, wherein xx is the sample number) is used as representative HIV1-O panel;
- BBI Performance Panel reference PRD201 (HIV1-M cultures diluted
20 in human plasma at about 10^5 copies per mL; sample numbers have the format 201-yy, wherein yy is the sample number) is used as representative HIV1-M panel;
- Negative controls: HIV1 negative plasma;
- Extraction: Kit Qiagen QiaAmp Viral RNA (reference 52904) used in
25 accordance with the manufacturer’s recommendations (volume of sample for extraction : 140 microliters);
- Primer pairs: HIV1-O primer pair (H1B5f, H1B13r, SEQ ID NO: 140, 168) and HIV1-M (H1B4f, H1B10r, SEQ ID NO: 7, 16) primer pair have each been used at 0.6 μ M per reaction;
- 30 - Probes: FAM-Dark Quencher probes (SH1BM5, SH1BO2, SEQ ID NO: 119, 191) at 0.6 μ M each per reaction;

- RT-PCR reagents: RNase-free water, and Qiagen QuantiTect Probe RT-PCR kit (Qiagen reference: 204443), used according to the manufacturer's instructions, $[MgCl_2] = 4 \text{ mM}$;
- Apparatus: IQ-Cycler Bio-Rad;
- 5 - Thermal cycling:
30' at 42°C,
15' at 95°C,
(15'' at 94°C – 30'' at 55°C – 30'' at 72°C) x 50,
+4°C.

10

The same RT-PCR procedure has been followed for both types of amplification experiments.

Interpretation of the results: for each assay, one determines a threshold cycle
15 (Ct) which is the level of fluorescence that is considered to be significantly above the background level of fluorescence measured in the early cycles of the amplification. The Ct value is inversely proportional to the concentration of target: the lower the Ct, the higher the concentration of target.

20 In the following tables:

CT= Threshold Cycle;

RFU max = maximal Relative Fluorescence Units observed at the end of the PCR run;

CTL - = negative control;

25 N/A= sample whose level of fluorescence is below the background level;

Avg.: average.

30

Table 4 below gives illustrative experimental results:

Sample	Group/ Subtype	HIV1-M primers and probe			HIV1-O primers and probe		
		CT		Avg	CT		Avg
201-1	A	37.6	38.3	38.0	44.3	N/A	
201-2	B	29.0	29.3	29.2	N/A	N/A	
201-3	C	37.5	38.8	38.2	N/A	N/A	
201-4	D	34.1	34.1	34.1	N/A	N/A	
201-5	E	32.1	35.2	33.7	N/A	N/A	
201-6	F	28.1	31.0	29.6	N/A	N/A	
201-7	G	31.9	32.0	32.0	N/A	N/A	
201-8	H	35.2	35.2	35.2	N/A	N/A	
201-9	CTL -	N/A	N/A		N/A	N/A	
301-1	O	N/A	N/A		32.4	31.8	32.1
301-2	O	41.1	N/A		33.0	32.8	32.9
301-3	O	N/A	N/A		30.9	31.0	31.0
301-4	O	N/A	47.5		N/A	35.7	35.7
Control (water)		N/A			N/A		
		N/A			N/A		
		N/A			N/A		

The results are illustrated by Figures 4A and 4B.

- 5 On Figure 4A, are shown the results obtained with the HIV1-M primers (H1B4f, H1B10r, SEQ ID NO: 7, 16) and HIV1-M probes (SH1BM5, SEQ ID NO: 119) of the invention on the HIV1-M panel (BBI PRD201), on the HIV1-O panel (BBI PRD301), and on the controls.

On Figure 4B, are shown the results obtained with the HIV1-O primers
 10 (H1B5f, H1B13r, SEQ ID NO: 140, 168) and HIV1-O probes (SH1BO2, SEQ

ID NO: 191) of the invention on the HIV1-O panel, on the HIV1-M panel, and on the controls.

In both cases, no amplification is detected in quantitative real-time RT-PCR when the HIV1-M primers and probes of the invention are used on HIV1-O
5 samples, and conversely.

Advantageously according to the invention, no cross-hybridization occurs between the HIV1-M amplicon and the HIV1-O probes, nor between the HIV1-O amplicon and the HIV1-M probes.

The HIV1-M and HIV1-O primers and probes according to the invention have
10 thus proven to allow for very high specificity detection in real-time quantitative RT-PCR conditions.

Example 3: HIV1-M and HIV1-O quantification

15 This example illustrates that the primers and probes of the invention allows for quantification of HIV viral charge by RT-PCR.

The primers and probes have the following sequences:

HIV1-M primers:

20 Forward: TTGGAGAGCAATGGCTAGTGA (H1B4f, SEQ ID NO: 7)
Reverse: TGTGTGCAATCTAGTTGCCATA (H1B10r, SEQ ID NO: 16)

HIV1-M probe:

ATAGTAGCCAGCTGTGATAAATGTC (SEQ ID NO: 110)

HIV1-O primers:

25 Forward: TTGGAGAGCACTAGCTAGTGA (H1B5f, SEQ ID NO: 140)
Reverse: TGTGTGCAATCTATTTGCCATA (H1B13r, SEQ ID NO: 168)

HIV1-O probe:

GAAATCATTGCTAGTTGTCCTAAATGTCATAT (SEQ ID NO: 190)

According to the invention, each probe is in this case produced as a molecular beacon, with “TGCGC” as target-unrelated arm in 5’, and with “GCGCA” as target-unrelated arm in 3’ (both arms are underlined). The 5’arm is labelled with a FAM fluorophore, and the 3’ arm with a Dark Quencher.

5

Probe sequences are thus as follows (beacon arms are shown underlined):

HIV1-M probe in a molecular beacon format:

FAM-TGCGCATAGTAGCCAGCTGTGATAAATGTCGCGCA-DQ
(SH1BM5, SEQ ID NO: 119)

10

HIV1-O probe in a molecular beacon format:

FAM-TGCGCGAAATCATTGCTAGTTGTCCTAAATGTCATATGCGCA-DQ
(SH1BO2, SEQ ID NO: 191)

Details of the RT-PCR procedure are as follows:

15

- Panels:

- BBI Performance Panel reference PRD301 (HIV1-O cultures diluted in human plasma at about 10^5 copies per mL; sample numbers have the format 301-xx, wherein xx is the sample number) is used as representative HIV1-O panel;

20

- BBI Performance Panel reference PRD201 (HIV1-M cultures diluted in human plasma at about 10^5 copies per mL; sample numbers have the format 201-yy, wherein yy is the sample number) is used as representative HIV1-M panel, and corresponds to several different genotypes of the HIV1-M subtype;

25

- BBI Accurun 315 panel series 500 (sub-type B) is used as a reference panel to produce the reference standard curve, constructed by plotting the log of known target concentrations against the corresponding Ct. The concentration of an unknown sample is then defined by mapping the corresponding Ct to the standard curve;

- Negative controls: HIV1 negative plasma;

30

- Extraction: Kit Qiagen QiaAmp Viral RNA (reference 52904) used in accordance with the manufacturer’s recommendations (volume of sample for extraction : 140 microliters);

- Primer pairs: HIV1-O primer pair (SEQ ID NO: 7, 8) and HIV1-M primer pair (SEQ ID NO: 1, 2) have each been used at 0.6 microM per reaction;
- Probes: FAM-Dark Quencher probes (SEQ ID NO: 4, 10), 0.2 microM each per reaction;
- RT-PCR reagents: RNase-free water, and Qiagen QuantiTect Probe RT-PCR kit (Qiagen reference: 204443), according to the manufacturer's instructions, [MgCl₂] 4mM;
- Apparatus: IQ-Cycler Bio-Rad;
- Thermal cycling:
 30' at 42°C (RT step),
 15' at 95°C (polymerase activation step),
 (15" at 94°C – 30" at 55°C – 30" at 72°C) x 50,
 + 4°C.

15

Two series of amplification experiments are performed with the primers and probes of the invention:

a) RT-PCR with a single pair of primers and probes (HIV1-M primer pair (H1B4f, H1B10r, SEQ ID NO: 7, 16) and HIV1-M probe (SH1BM5, SEQ ID NO: 119);

b) RT-PCR with both pairs of primers and both probes (HIV1-M primer pair (H1B4f, H1B10r, SEQ ID NO: 7, 16) + HIV1-O primer pair (H1B5f, H1B13r, SEQ ID NO: 140, 168) and HIV1-M probe (SH1BM5, SEQ ID NO: 119) + HIV1-O probe (SH1BO2, SEQ ID NO: 191)).

25

The Ct results of each assay are used for the quantification of panels PRD201 and PRD301 by mapping the corresponding Ct to the standard curve. The quantification values corresponding to the number of RNA copies/ml in each pure sample are compared with commercially-available kits used in accordance with the manufacturers' recommendations: kit Amplicor HIV1 Monitor Version 1.5 from Roche (ref 87674), kit Quantiplex HIV1 RNA 3.0

30

bDNA from Bayer (ref 6147) and kit Nuclisens HIV-1 QT from Organon Teknika (ref 84152).

Representative results are shown in Tables 5 and 6:

5

Table 5 (experiment a): Results obtained a single pair of primers (HIV1-M primer pair, H1B4f, H1B10r, SEQ ID NO: 7, 16) and a single probe (HIV1-M beacon probe, SEQ ID NO: 119) on the HIV1-M sample panel

Sample	Genotype	Results copies per mL				
		Roche Amplicor	Bayer Quantiplex	Organon Nuclisens	Invention	
					CT	Avg.
201-1	A	4.00E+03	3.00E+03	2.00E+03	37.8	2.80E+03
201-2	B	2.00E+05	8.00E+04	2.00E+05	29.2	4.30E+04
201-3	C	2.00E+03	5.00E+03	1.00E+04	36.8	3.90E+03
201-4	D	2.00E+05	1.00E+05	3.00E+05	30.9	2.50E+04
201-5	E	2.00E+05	9.00E+04	6.00E+04	33.9	9.80E+03
201-6	F	5.00E+05	9.00E+04	2.00E+05	28.7	5.00E+04
201-7	G	5.00E+05	8.00E+04	1.00E+05	32.3	1.60E+04
201-8	H	2.00E+05	3.00E+04	9.00E+04	34.7	7.60E+03
201-9	Diluent	ND	ND	ND	N/A	

10

It is apparent from these results that the HIV1-M primers and probes of the invention allow for an accurate real-time quantitative detection of all HIV1-M genotypes, and good correlation with the commercially available kits.

15

Table 6 (experiment b): Results obtained with HIV1-M and HIV1-O primers (H1B4f, H1B10r, H1B5f, H1B13r, SEQ ID NO: 7, 16, 140, 168) and HIV1-M and HIV1-O probes (SEQ ID NO: 119, 191) on the HIV1-O sample panel

5

Sample	Genotype	Results copies per mL				
		Roche Amplicor	Bayer Quantiplex	Organon Nuclisens	Invention	
					CT	Avg.
301-1	O	ND	3.00E+02	< LDL	32.1	1.60E+04
301-2	O	ND	2.00E+03	< LDL	32.9	1.00E+04
301-3	O	ND	8.00E+02	< LDL	31	1.40E+04
301-4	O	ND	3.00E+02	< LDL	35.7	2.00E+03
301-5	Diluent	ND	ND	ND	N/A	

It can be seen from Table 6 that the higher level of quantification on HIV1-O panel is obtained with both HIV1-M and HIV1-O primers and probes of the invention. (<LDL = less than lower detection limit).

Example 4: HIV1/HIV2 Multiplex assay (Figures 5A and 5B)

This example illustrates that the HIV1 and HIV2 primers and probes of the invention may be used in a multiplex assay for HIV1 or HIV2 signal. It also demonstrates the possibility to follow the fluorescence of two targets in the same tube by use of two different fluorophores (FAM and ROX).

The primers and probes have the following sequences:

20 HIV1-M primers:

Forward: TTGGAGAGCAATGGCTAGTGA (H1B4f, SEQ ID NO: 7)

Reverse: TGTGTGCAATCTAGTTGCCATA (H1B10r, SEQ ID NO: 16)

HIV1-M probe:

ATAGTAGCCAGCTGTGATAAATGTC (SEQ ID NO: 110)

HIV2 forward primer (H2A3f, SEQ ID NO: 195):

AGGAAGRCARACAGCACTCTTC with R=A/G (T_m = 66°C)

HIV2 reverse primer (H2A3r, SEQ ID NO: 250):

5 GGTACTCCRAAGGGGTTTGTCTAT with R=A/G (T_m = 65°)

HIV2-A probe (HIV2-A probe, SEQ ID NO: 297):

GGCCAATAACACACTTGCACACA

10 According to the invention, each probe is in this case produced as a molecular beacon, with “CGCGC” as target-unrelated arm in 5’, and with “GCGCG” as target-unrelated arm in 3’ (both arms are underlined). The 5’arm is labelled with a FAM or ROX fluorophore, and the 3’ arm with a Dabcyl moiety.

Probe sequences are thus as follows (beacon arms are shown underlined):

15 HIV1-M probe in a molecular beacon format:

FAM-CGCGCATAGTAGCCAGCTGTGATAAATGTCGCGCG-Dabcyl
(SH1BM10, SEQ ID NO: 128)

HIV2-A probe in a molecular beacon format:

20 ROX - CGCGCGGGCCAATAACACACTTGCACACAGGCGCG – Dabcyl
(HIV2-A beacon probe, SH2A14a, SEQ ID NO: 299)

Details of the RT-PCR procedure are as follows:

- HIV2-A positive sample: obtained from infected patient (Centre Hospitalier Bichat, Laboratory of Virology, Assistance Publique –
25 Hôpitaux de Paris, France);
- BBI Accurun 315 panel series 500 (sub-type B);
- Negative controls: HIV1 and HIV2 negative sample;
- Extraction: Kit Qiagen QiaAmp Viral RNA (reference 52904) used in
accordance with the manufacturer’s recommendations (volume of
30 sample for extraction : 140 microliters);
- Primer pairs: HIV1-M primer pair (SEQ ID NO: 7, 16) and HIV2
primer pair (SEQ ID NO: 195, 250) have been used respectively at 0.5

microM each per reaction for HIV1-M and 0.3 microM each per reaction for HIV2;

- Probes: HIV1-M FAM-Dabcyl probe (SEQ ID NO: 128) and HIV2-A ROX Dabcyl probe (SEQ ID N° 299), 0.4 microM each per reaction;
- 5 - RT-PCR reagents: RNase-free water, and Qiagen QuantiTect Probe RT-PCR kit (Qiagen reference: 204443), according to the manufacturer's instructions, [MgCl₂] 4mM;
- Apparatus: IQ-Cycler Bio-Rad;
- Thermal cycling:
 10 30' at 42°C (RT step),
 15' at 95°C (polymerase activation step),
 (15'' at 94°C – 30'' at 55°C – 30'' at 72°C) x 50,
 + 4°C.

15 Two series of amplification experiments have been performed with the primers and probes of the invention:

- a) RT-PCR with a single pair of primers and probe HIV1-M (H1B4f, H1B10r, SH1BM10, SEQ ID NO: 7, 16, 119, a single pair of primers and probe HIV2 (H2A3f, H2A3r, SH2A14a, SEQ ID NO: 195, 250, 299) with a range of HIV1-M target concentrations.
- 20 b) RT-PCR with a single pair of primers and probe HIV1-M (H1B4f, H1B10r, SH1BM10, SEQ ID NO: 7, 16, 119), a single pair of primers and probe HIV2 (H2A3f, H2A3r, SH2A14a, SEQ ID NO: 195, 250, 299) with a range of HIV2 target dilutions.

25

Interpretation of the results: for each assay one determines a threshold cycle (Ct) which is the level of fluorescence that is considered to be significantly above the background level of fluorescence measured in the early cycles of the amplification. The Ct value is inversely proportional to the concentration of target: the lower the Ct, the higher the concentration of target.

30

In the following tables, CT= Threshold Cycle; RFU max = maximal Relative Fluorescence Units observed at the end of the PCR run; CTL - = negative control; N/A= sample whose level of fluorescence is below the background level; Avg = average; cop = copies.

5

Illustrative Ct results are shown in Table 7:

HIV1-M				HIV2-A			
Number cop/PCR	CT	CT	Avg	Dilution	CT	CT	Avg
10000	28.2	28.2	28.2	1/300	28.0	27.7	27.9
1000	31.6	31.3	31.5	1/3000	31.9	32.4	32.2
100	35.7	35.7	35.7	1/30000	36.6	36.7	36.7
10	39.4	N/A	39.4	1/300000	40.7	N/A	40.7
CTL - (water)	N/A	N/A		CTL - (water)	N/A	N/A	

Its is apparent from these results that the HIV1 and HIV2 primers and probe of the invention may be used in a multiplex assay for the detection of HIV1-M or HIV2 target.

10

The results are illustrated by figures 5A and 5B (for each dilution, there are 2 curves since experiments are here carried out in duplicate).

Example 5: HIV2 / IC Multiplex assay (Figures 6A and 6B)

15

This example illustrates that the HIV2 target and internal control (IC) may be co-amplified using the HIV2 primers and probes of the invention and selected IC primers and probe without any perturbation of the HIV2 signal. It also demonstrates the possibility to follow the fluorescence of two targets in the same tube by use of two different fluorophores (ROX and TAMRA)

20

This example involves the use of one pair of HIV2 primers and one HIV2 probe. These primers and probes target a 147 bp sequence in HIV2 isolates.

They have the following sequences:

HIV2 forward primer (H2A3f, SEQ ID NO: 195):

5 AGGAAGRCARACAGCACTCTTC with R=A/G ($T_m = 66^\circ\text{C}$)

HIV2 reverse primer (H2A3r, SEQ ID NO: 250):

GGTACTCCRAAGGGGTTTGTCTAT with R=A/G ($T_m = 65^\circ$)

HIV2-A probe (HIV2-A probe, SEQ ID NO: 297):

GGCCAATAACACACTTGACACACA

10

This example also involves the use of one pair of IC primers and one IC probe, selected to get the same amplified fragment size and GC% than the HIV target.

15 Each of these probes is used as a molecular beacon in this example. The target-unrelated beacon arms which have been added at each end of the HIV2 probe is shown underlined (ROX = fluorophore; Dabcyl = quencher).

HIV2-A probe in molecular beacon format (HIV2-A beacon probe, SH2A14a, SEQ ID NO: 299):

20 ROX - CGCGCGGCCAATAACACACTTGACACAGCGCG – Dabcyl

For IC probe, TAMRA has been chosen as fluorophore and Dabcyl as quencher.

25 Two series of real-time quantitative amplification experiments have been performed:

a) HIV2 primer pair + HIV2-A beacon probe (H2A3f, H2A3r, SH2A9a, SEQ ID NO: 195, 250, 299) + IC primers pair + IC beacon probe with addition of HIV2 target alone;

30 b) HIV2 primer pair + HIV2-A beacon probe (H2A3f, H2A3r, SH2A9a, SEQ ID NO: 195, 250, 299) + IC primers pair + IC beacon probe with addition of HIV2 target and IC.

Details of the procedure are as follows:

- HIV2-A positive sample: obtained from infected patient (Centre Hospitalier Bichat, Laboratory of Virology, Assistance Publique – Hôpitaux de Paris, France);
- 5 - IC dilution: IC is diluted in order to obtain 10^6 cop/PCR;
- Negative controls: HIV2-negative plasma;
- Nucleic acid extraction: Kit Qiagen QiaAmp Viral RNA (reference 52904) used in accordance with the manufacturer's recommendations (volume of sample for extraction = 140 microliters);
- 10 - Primer pair: 0.3 microM each per reaction (SEQ ID NO: 11, 12 and IC primers);
- Probes: HIV2-A Rox-Dabcyl beacon probe as described above (SEQ ID n° 299) and IC Tamra-Dabcyl beacon probe, 0,4 microM each per reaction;
- 15 - RT-PCR reagents: RNase-free water, and Qiagen QuantiTect Probe RT-PCR kit (Qiagen reference: 204443) used according to the manufacturer's instructions, $[MgCl_2] = 4$ mM;
- Apparatus: IQ-Cycler Bio-Rad;
- Thermal cycling:
- 20 30' at 42°C (RT step),
- 15' at 95°C (polymerase activation step),
- (30'' at 94°C – 30'' at 55°C – 30'' at 72°C) x 50,
- +4°C.

25 HIV2-A positive sample was submitted to nucleic acid extraction and, after dilution to 1/300, 1/3000 and 1/30000 in water to RT-PCR amplification as described in experiments **a)** and **b)**.

IC dilution was submitted to RT-PCR amplification (around 10^6 cop/PCR) as described in experiment **b)**. (cop= copies).

30

Interpretation of results : for each assay is determined a threshold cycle (Ct) which is the level of fluorescence that is considered to be significantly above

the background level of fluorescence measured in the early cycles of the amplification. The Ct value is inversely proportional to the concentration of target: the lower the Ct, the higher the concentration of target.

- 5 In the following tables, CT= Threshold Cycle; max RFU = maximal Relative Fluorescence Units observed at the end of the PCR run; CTL- = negative control; N/A= sample whose level of fluorescence is below the background level.
- 10 Table 8: CT values obtained with HIV2 primer pair + HIV2-A beacon probe + IC primer pair + IC beacon probe in experiments a) and b) (H2A3f, H2A3r, SH2A14a, SEQ ID NO: 195, 250, 299 for HIV2) when fluorescence of ROX is read (HIV2 signal)

Assay conditions	HIV2-A 1/300				HIV2-A 1/3000				HIV2-A 1/30000			
	CT	CT	CT	Avg	CT	CT	CT	Avg	CT	CT	CT	Avg
Exp. a) : HIV2 target	25.3	25.4	25.7	25.5	28.8	28.2	28.8	28.6	32.3	33.0	32.7	32.7
Exp. b) : HIV2 target + IC	25.4	25.3	25.4	25.4	29.0	20.3	28.7	29.0	33.0	34.1	34.8	34.0

15

It can be seen from this table that the HIV2 Ct values did not change with addition of IC, irrespective of the dilution of HIV2-A extracted sample tested (1/300, 1/3000, 1/30000).

- 20 Table 9: CT values obtained with HIV2 primer pair + HIV2-A beacon probe + IC primer pair + IC beacon probe in experiment b) (H2A3f, H2A3r, SH2A14a,

SEQ ID NO: 195, 250, 299 for HIV2) when fluorescence of TAMRA is read (IC signal)

Assay conditions	IC											
	HIV2-A 1/300				HIV2-A 1/3000				HIV2-A 1/30000			
	CT	CT	CT	Avg	CT	CT	CT	Avg	CT	CT	CT	Avg
Exp. b) : IC + HIV2 target	28.5	28.7	28.7	28.6	27.2	27.7	28.0	27.6	27.7	27.7	27.7	27.7

- 5 It can be seen from this table that the IC Ct values are very reproducible irrespective of the dilution of HIV2-A extracted sample added.

These results are illustrated by Figures 6A, 6B and 6C (for each dilution of HIV2-A, there are 3 curves since experiments are here carried out in triplicate in Figures 6A and 6B; there are 6 curves for the negative control; and there are 9 curves for the IC, all with the same concentration on Figure 6C).

10

HIV vend3mai05.ST25
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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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23

HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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HIV vend3mai05.ST25

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<212> DNA

<213> Artificial Sequence

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atagaacggt actccgaagg wttgttctat

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<210> 287

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<212> DNA

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HIV vend3mai05.ST25

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<213> Artificial Sequence

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HIV vend3mai05.ST25

<220>

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30

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30

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HIV vend3mai05.ST25

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30

<210> 296

<211> 30

<212> DNA

<213> Artificial Sequence

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<223> oligonucleotide for detection

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<210> 297

<211> 23

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<213> Artificial Sequence

<220>

<223> oligonucleotide for detection

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23

<210> 298

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> oligonucleotide for detection

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agcgcggcca ataacacact tgacacagc gct

33

<210> 299

HIV vend3mai05.ST25

<211> 33

<212> DNA

<213> Artificial Sequence

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<223> oligonucleotide for detection

<400> 299

cgcgcgcca ataacacact tgcacacagc gcg

33

<210> 300

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22

<210> 301

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32

<210> 302

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<400> 302

cgcgcgcta tcacacacct gcacacagcg cg

32

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HIV vend3mai05.ST25

<211> 32

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<223> oligonucleotide for detection

<400> 303

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32

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<223> n is inosine (i)

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cgcgcgccn atnacacacn tgcacacagc gcg

33

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<211> 254

HIV vend3mai05.ST25

<212> DNA

<213> Human immunodeficiency virus

<400> 305

ggaggaaatg	aacaagtaga	taaattagtc	agtgctggaa	tcaggaaagt	actattttta	60
gatggaatag	ataaggccca	agatgaacat	gagaaatatc	acagtaattg	gagagcaatg	120
gctagtgtg	ttaacctgcc	acctgtagta	gcaaaagaaa	tagtagccag	ctgtgataaa	180
tgtcagctaa	aaggagaagc	catgcatgga	caagtagact	gtagtccagg	aatatggcaa	240
ctagattgta	caca					254

<210> 306

<211> 149

<212> DNA

<213> Human immunodeficiency virus

<400> 306

aattggagag	caatggctag	tgattttaac	ctgccacctg	tagtagcaaa	agaaatagta	60
gccagctgtg	ataaatgtca	gctaaaagga	gaagccatgc	atggacaagt	agactgtagt	120
ccaggaatat	ggcaactaga	ttgtacaca				149

<210> 307

<211> 147

<212> DNA

<213> Human immunodeficiency virus

<400> 307

ttggagagca	atggctagtg	attttaacct	gccacctgta	gtagcaaaag	aaatagtagc	60
cagctgtgat	aaatgtcagc	taaaaggaga	agccatgcat	ggacaagtag	actgtagtcc	120
aggaatatgg	caactagatt	gtacaca				147

<210> 308

<211> 149

<212> DNA

<213> Human immunodeficiency virus

<400> 308

ttggagagca	atggctagtg	attttaacct	gccacctgta	gtagcaaaag	aaatagtagc	60
cagctgtgat	aaatgtcagc	taaaaggaga	agccatgcat	ggacaagtag	actgtagtcc	120
aggaatatgg	caactagatt	gtacacatt				149

<210> 309

<211> 254

<212> DNA

HIV vend3mai05.ST25

<213> Human immunodeficiency virus

<400> 309

ggaggaaatg	aaaaaataga	taaattagta	agcaaggata	ttagaagagt	cctgttccta	60
gaaggaatag	accaggcaca	agaagatcat	gaaaaatatac	atagtaattg	gaaagcacta	120
gctagtgaat	ttggactacc	accagtgggtg	gccaaggaaa	tcattgctag	ctgtcctaaa	180
tgtcatataa	aaggggaagc	aattcatggt	caggtagact	gcagtccaga	agtatggcaa	240
atagattgca	caca					254

<210> 310

<211> 149

<212> DNA

<213> Human immunodeficiency virus

<400> 310

aattggaaag	cactagctag	tgaatttgga	ctaccaccag	tggtggccaa	ggaaatcatt	60
gctagctgtc	ctaaatgtca	tataaaaggg	gaagcaattc	atggtcaggt	agactgcagt	120
ccagaagtat	ggcaaataga	ttgcacaca				149

<210> 311

<211> 147

<212> DNA

<213> Human immunodeficiency virus

<400> 311

ttggaaagca	ctagctagt	aatttggact	accaccagt	gtggccaagg	aaatcattgc	60
tagctgtcct	aaatgtcata	taaaagggga	agcaattcat	ggtcaggtag	actgcagtcc	120
agaagtatgg	caaatagatt	gcacaca				147

CLAIMS

1. A process for the detection of at least one HIV in a sample, comprising the step of producing at least one amplicon from said sample by means of at least two oligonucleotides, wherein said at least two oligonucleotides are primers, which are structurally suitable for annealing to the K03455 HIV1-M reference isolate, or to the L20587 HIV1-O reference isolate, or to the M30502 HIV2 reference isolate at such positions that they amplify from said HIV reference isolate a polynucleotide, the sequence of which consists of a sequence selected from the group consisting of:
 - SEQ ID NO: 2 and the fragments of SEQ ID NO: 2 selected from SEQ ID NO: 306, SEQ ID NO: 307 and SEQ ID NO: 308;
 - SEQ ID NO: 4 and the fragments of SEQ ID NO: 4 selected from SEQ ID NO: 310, SEQ ID NO: 311;
 - SEQ ID NO: 5;
 - SEQ ID NO: 1 and the fragment thereof of SEQ ID NO: 305;
 - and
 - SEQ ID NO: 3 and the fragment thereof of SEQ ID NO: 309;and optionally, comprising the step of detecting said amplicon by means of at least one probe, wherein said at least one HIV is detected.
2. The process according to Claim 1, wherein said sequence is SEQ ID NO: 2 or one of said fragments thereof, and wherein said process is carried out with at least one oligonucleotide selected from the group consisting of SEQ ID NO: 9, 12, 14, 15, 24, 52, 82, 91, 100, 109, 127 and 136.
3. The process according to Claim 2, wherein said process is carried out with at least one primer selected from the group consisting of SEQ ID NO: 9, 12, 14, 15, 24, 52 and 82.

4. The process according to any one of Claims 2-3, wherein said process is carried out with at least one probe selected from the group consisting of SEQ ID NO: 91, 100, 109, 127 and 136.
5. The process according to any one of Claims 2-4, wherein said process is carried out with at least one primer selected from the group consisting of:
 - SEQ ID NO: 9, 12, 14 and 15;and with at least one primer selected from the group consisting of:
 - SEQ ID NO: 24, 52 and 82;and, optionally, with at least one probe selected from the group consisting of SEQ ID NO: 91, 100, 109, 118, 127 and 136.
6. The process according to Claim 1, wherein said sequence is SEQ ID NO:4 or one of said fragments thereof, and wherein said process is carried out with at least one oligonucleotide selected from the group consisting of SEQ ID NO: 148, 157, 167, 170, 179, 189 and 190 to 194.
7. The process according to Claim 6, wherein said process is carried out with at least one primer selected from the group consisting of SEQ ID NO: 148, 157, 167, 170, 179 and 189.
8. The process according to any one of Claims 6-7, wherein said process is carried out with at least one probe selected from the group consisting of:
 - SEQ ID NO: 190 to 194.
9. The process according to any one of Claims 6-8, wherein said process is carried out with at least one primer selected from the group consisting of:
 - SEQ ID NO: 148, 157 and 167;

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and with at least one primer selected from the group consisting of:

- SEQ ID NO: 170, 179 and 189;

and, optionally, with at least one probe selected from the group consisting of:

- SEQ ID NO: 190 to 194.

10. The process according to Claim 1, wherein said sequence is SEQ ID NO: 5, and wherein said process is carried out with at least one oligonucleotide selected from the group consisting of SEQ ID NO: 221, 249, 276, 287, 296 and 297 to 303.

11. The process according to Claim 10, wherein said process is carried out with at least one primer selected from the group consisting of SEQ ID NO: 221, 249, 276, 287 and 296.

12. The process according to any one of Claims 10-11, wherein said process is carried out with at least one probe selected from the group consisting of SEQ ID NO: 297 to 303.

13. The process according to any one of Claims 10-12, wherein said process is carried out with at least one primer selected from the group consisting of:

- SEQ ID NO: 221 and 249;

and with at least one primer selected from the group consisting of:

- SEQ ID NO: 276, 287 and 296;

and, optionally, with at least one probe selected from the group consisting of SEQ ID NO: 297 to 303.

14. The process according to Claim 1, wherein said sequence is SEQ ID NO: 1 or said fragment thereof, and wherein said process is carried out with at least one oligonucleotide selected from the group consisting of SEQ ID NO: 6, 24, 52, 82, 91, 100, 109, 127 and 136.

15. The process according to Claim 14, wherein said process is carried out with at least one primer selected from the group consisting of SEQ ID NO: 6, 24, 52 and 82.
16. The process according to any one of Claims 14-15, wherein said process is carried out with at least one probe selected from the group consisting of SEQ ID NO: 91, 100, 109, 127 and 136.
17. The process according to any one of Claims 14-16, wherein said process is carried out with at least one primer selected which is:
 - SEQ ID NO: 6;and with at least one primer selected from the group consisting of:
 - SEQ ID NO: 24, 52 and 82;and, optionally, with at least one probe selected from the group consisting of SEQ ID NO: 91, 100, 109, 118, 127 and 136.
18. The process according to Claim 1, wherein said sequence is SEQ ID NO: 3 or said fragment thereof, and wherein said process is carried out with at least one oligonucleotide selected from the group consisting of SEQ ID NO: 139, 170, 179, 189 and 190 to 194.
19. The process according to Claim 18, wherein said process is carried out with at least one primer selected from the group consisting of SEQ ID NO: 139, 170, 179 and 189.
20. The process according to any one of Claims 18-19, wherein said process is carried out with at least one probe selected from the group consisting of SEQ ID NO: 190 to 194.
21. The process according to any one of Claims 18-20, wherein said process is carried out with at least one primer which is:
 - SEQ ID NO: 139;and with at least one primer selected from the group consisting of:

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- SEQ ID NO: 170, 179 and 189;

and, optionally, with at least one probe selected from the group consisting of SEQ ID NO: 190 to 194.

22. The process according to any one of Claims 1-21, wherein said step of producing at least one amplicon comprises at least one quantitative and/or qualitative, multiplex and/or simplex PCR amplification.

23. The process according to any one of Claims 1-22, wherein said oligonucleotides are loop primers.

24. The process according to any one of Claims 1-23, wherein said oligonucleotides are loop primers, which comprise a 5' end tail of 3 to 7 nucleotides that is complementary to its 3' end.

25. The process of any one of claims 1-24, wherein said at least one HIV is selected from HIV1-M, HIV1-O, HIV2-A and HIV2-B.

26. The process of any one of claims 1-25, wherein said at least one HIV is selected from the group consisting of HIV1-M and HIV1-O, and wherein said amplification is a RT-PCR performed in accordance with the following thermal cycling:

30' at 42°C;

15' at 95°C;

[15'' at 94°C, 30'' at 55°C and 30'' at 72°C] x 50; and

+4°C.

27. The process of any one of claims 1-25, wherein said at least one HIV is selected from the group consisting of HIV2-A and HIV2-B, and wherein said amplification is a RT-PCR performed in accordance with the following thermal cycling:

30' at 42°C;

15' at 95°C;

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[30'' at 94°C, 30'' at 55°C and 30'' at 72°C] x 50; and
+4°C.

28. A polynucleotide, the sequence of which consists of a sequence selected from the group consisting of:

- SEQ ID NO: 2 and the fragments of SEQ ID NO: 2 selected from SEQ ID NO: 306, SEQ ID NO: 307, SEQ ID NO: 308;
- SEQ ID NO: 4 and the fragments of SEQ ID NO: 4 selected from SEQ ID NO: 310, SEQ ID NO: 311;
- SEQ ID NO: 5;
- SEQ ID NO: 1 and the fragment of SEQ ID NO: 305; and
- SEQ ID NO: 3 and the fragment of SEQ ID NO: 309.

29. An oligonucleotide, the sequence of which consists of a sequence selected from the group consisting of SEQ ID NO: 6, 8, 11, 14, 15, 24, 52, 82, 91, 100, 109, 127, 136, 139, 148, 157, 167, 170, 179, 189, 190 to 194, 221, 249, 276, 287, 296 and 297 to 303.

30. An oligonucleotide according to Claim 29, the sequence of which consists of a sequence selected from the group consisting of SEQ ID NO: 6, 8, 11, 14, 15, 24, 52, 82, 91, 100, 109, 127 and 136.

31. An oligonucleotide according to Claim 29, the sequence of which consists of a sequence selected from the group consisting of SEQ ID NO: 139, 148, 157, 167, 170, 179, 189 and 190 to 194.

32. An oligonucleotide according to Claim 29, the sequence of which consists of a sequence selected from the group consisting of SEQ ID NO: 221, 249, 276, 287, 296 and 297 to 299.

33. An oligonucleotide according to Claim 29, the sequence of which consists of a sequence selected from the group consisting of SEQ ID NO: 221, 249, 276, 287, 296 and 300 to 303.

34. An oligonucleotide according to any one of Claims 29-33, wherein said oligonucleotide is fluorescently labelled.

35. A set of oligonucleotides comprising at least two oligonucleotides selected from two different groups among the following groups:

- the group consisting in SEQ ID NO: 6, 9, 12, 14 and 15;
- the group consisting in SEQ ID NO: 24, 52 and 82; and
- the group consisting in SEQ ID NO: 91, 100, 109, 118, 127 and 136.

36. A set of oligonucleotides comprising at least one oligonucleotide selected from one the following groups:

- the group consisting of SEQ ID NO: 139, 148, 157 and 167;
- the group consisting of SEQ ID NO: 170, 179 and 189; and
- the group consisting of SEQ ID NO: 190 to 194.

37. A set of oligonucleotides comprising at least one oligonucleotide selected from one the following groups:

- the group consisting of SEQ ID NO: 221 and 249;
- the group consisting of SEQ ID NO: 276, 287 and 296; and
- the group consisting of SEQ ID NO: 297 to 299.

38. A set of oligonucleotides comprising at least one oligonucleotide selected from one the following groups:

- the group consisting of SEQ ID NO: 221 and 249;
- the group consisting of SEQ ID NO: 276, 287 and 296; and
- the group consisting of SEQ ID NO: 300 to 303.

39. A set of oligonucleotides comprising at least one oligonucleotide selected from one the following groups:

- the group consisting of SEQ ID NO: 91, 100, 109, 118, 127 and 136;

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- the group consisting of SEQ ID NO: 190 to 194;
- the group consisting of SEQ ID NO: 297 to 299; and
- the group consisting of SEQ ID NO: 300 to 303.

40. The set of oligonucleotides of any one of Claims 36-39, which comprises at least two oligonucleotides selected from two different groups among said groups.

41. A set of oligonucleotides according to any one of Claims 35-40, wherein at least one of said oligonucleotides is fluorescently labelled.

42. An amplicon, the nucleotide length of which is identical to the nucleotide length of a polynucleotide of claim 28, wherein said amplicon is obtained from a HIV selected from the group consisting of HIV1-M, HIV1-O, HIV2-A and HIV2-B and wherein said amplicon comprises a first sequence having a percentage of nucleotide identity of at least 90% with a second sequence, over the entire length of said second sequence, wherein said second sequence is selected from the group consisting of: SEQ ID NO: 91, 100, 109, 118, 127, 136, 190, 191, 192, 193, 194, 297, 298, 299, 300, 301, 302, 303 and the sequences, which are complementary to these SEQ ID NO: sequences over the entire length of these SEQ ID NO: sequences.

43. A composition comprising an amplicon according to claim 42 and at least one amplification reagent.

44. A polynucleotide, the nucleotide length of which is identical to the nucleotide length of a polynucleotide of claim 28, and which comprises a first sequence having a percentage of nucleotide identity of at least 90% with a second sequence, over the entire length of said second sequence, wherein said second sequence is selected from the group consisting of: SEQ ID NO: 91, 100, 109, 118, 127, 136, 190, 191, 192, 193, 194, 297, 298, 299, 300, 301, 302, 303 and the sequences, which are complementary

to these SEQ ID NO: sequences over the entire length of these SEQ ID NO: sequences.

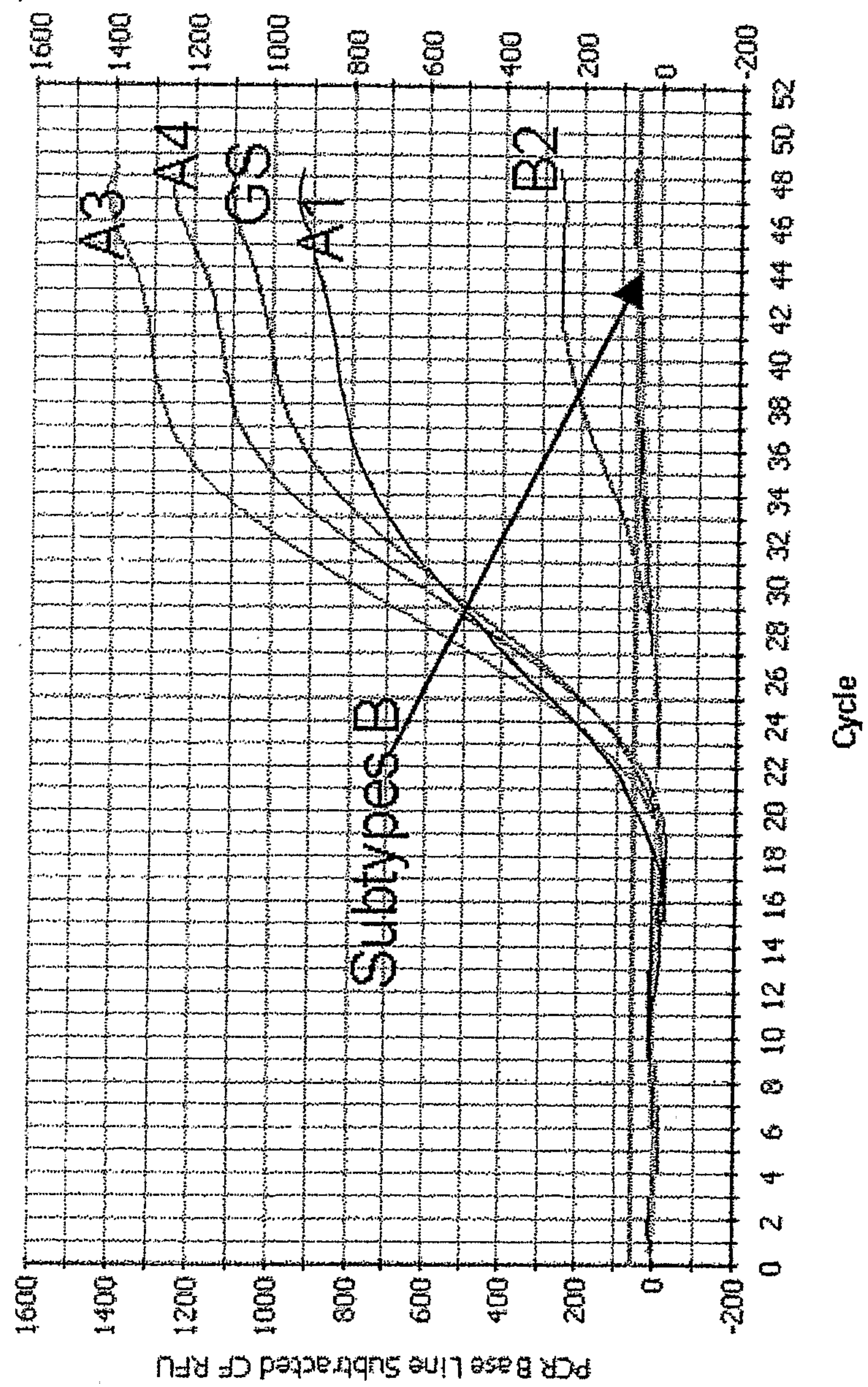
45. A kit for HIV diagnosis, which comprises at least one oligonucleotide according to any one of Claims 29-34 and instructions for use thereof.

46. A kit for HIV diagnosis comprising at least one set of oligonucleotides according to any one of Claims 35-41.

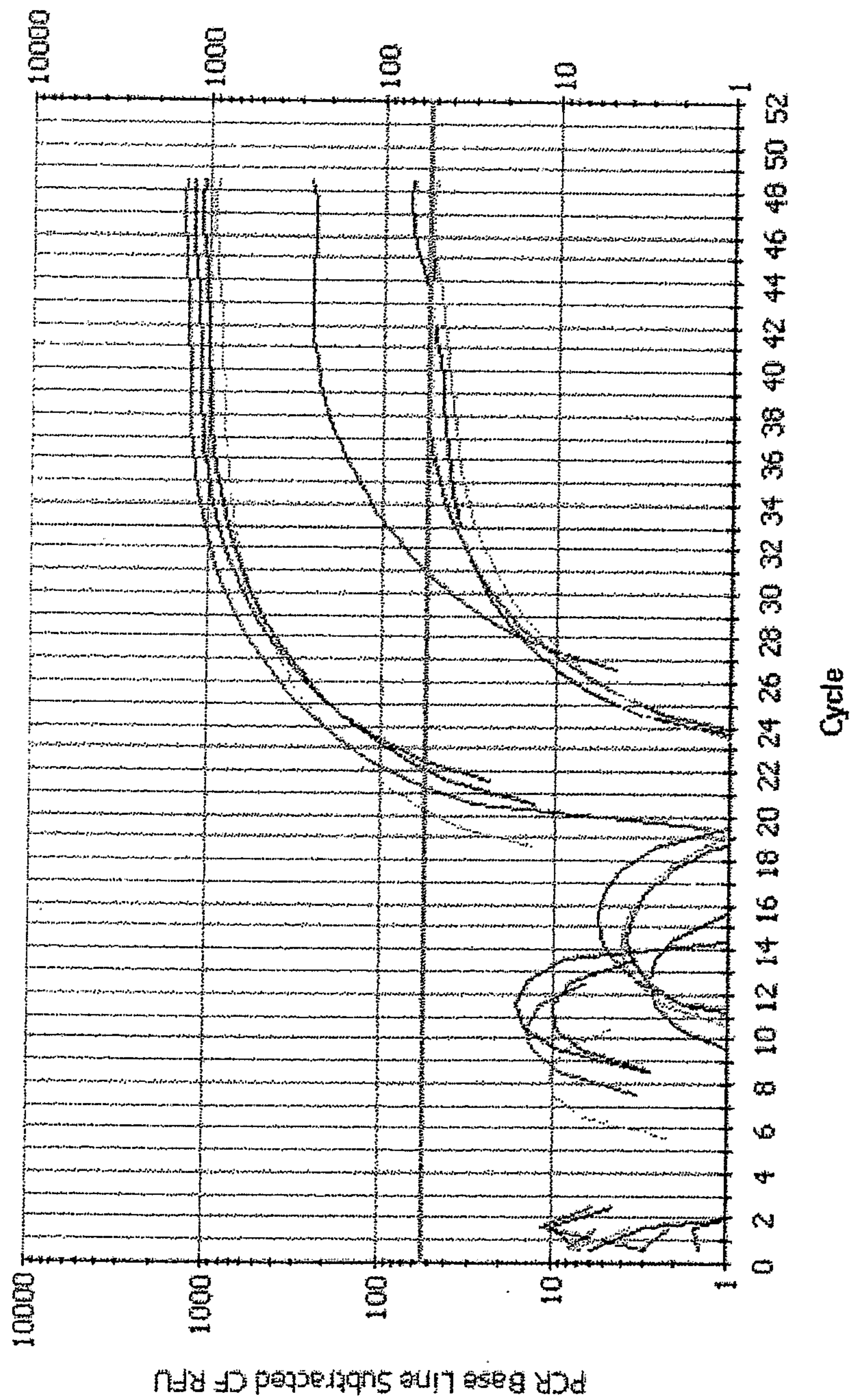
47. A kit for HIV diagnosis comprising at least one oligonucleotide according to any one of Claims 29-34, and at least one set of oligonucleotides according to any one of Claims 35-41.

48. The kit of any one of claims 45-47, which further comprises one or several elements selected from the group consisting of water, nuclease-free water, RNase-free water, DNase-free water, PCR-grade water, salts, magnesium, potassium, buffers, Tris buffer, enzymes, polymerases, reverse transcriptase, nucleotides, deoxynucleotides, dideoxynucleotides, dNTPs, dATP, dTTP, dCTP, dGTP, dUTP, DTT and/or RNase inhibitors, polynucleotides, polyT polynucleotides and polydT polynucleotides.

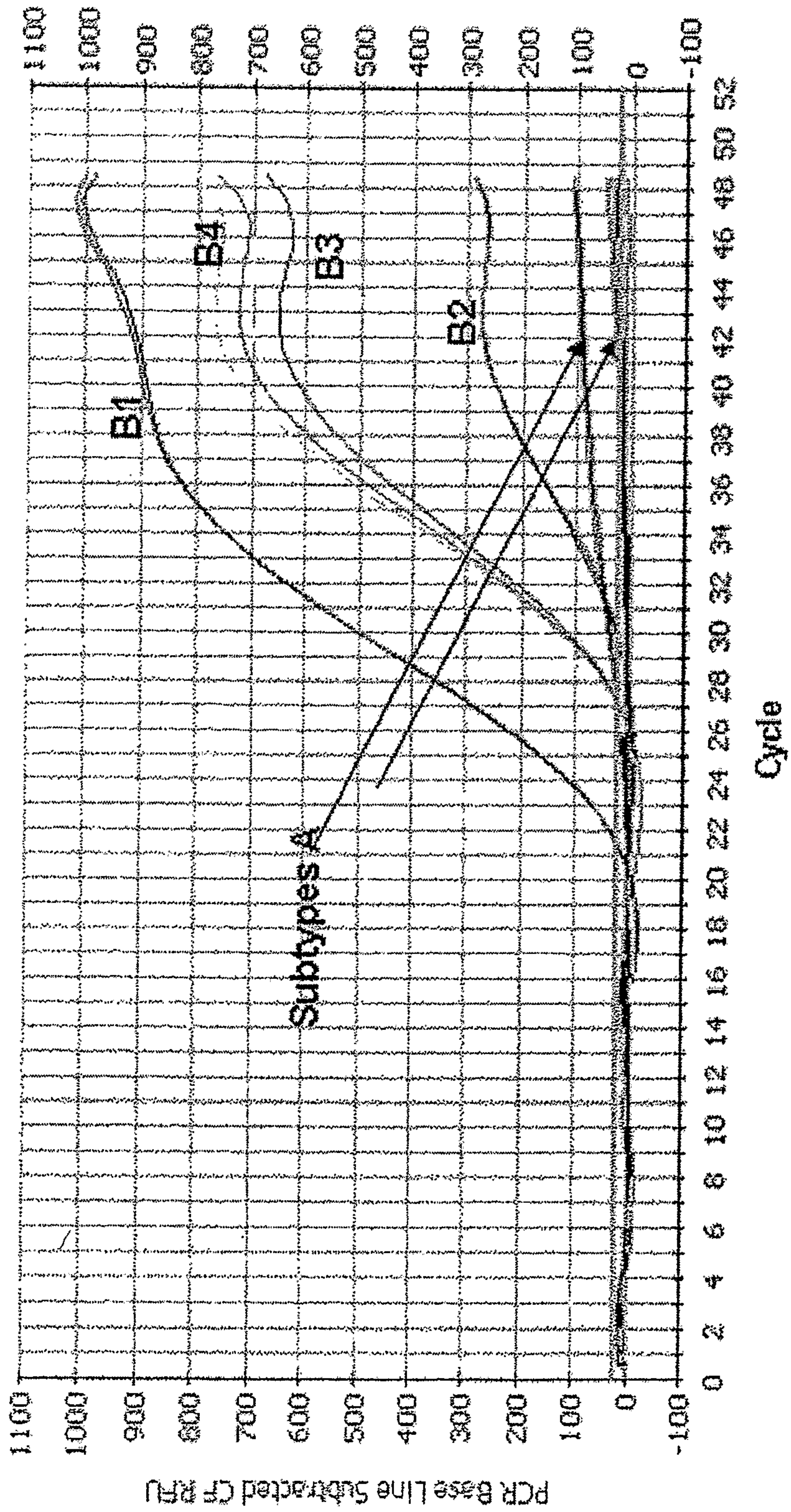
1/25

**Figure 1A**

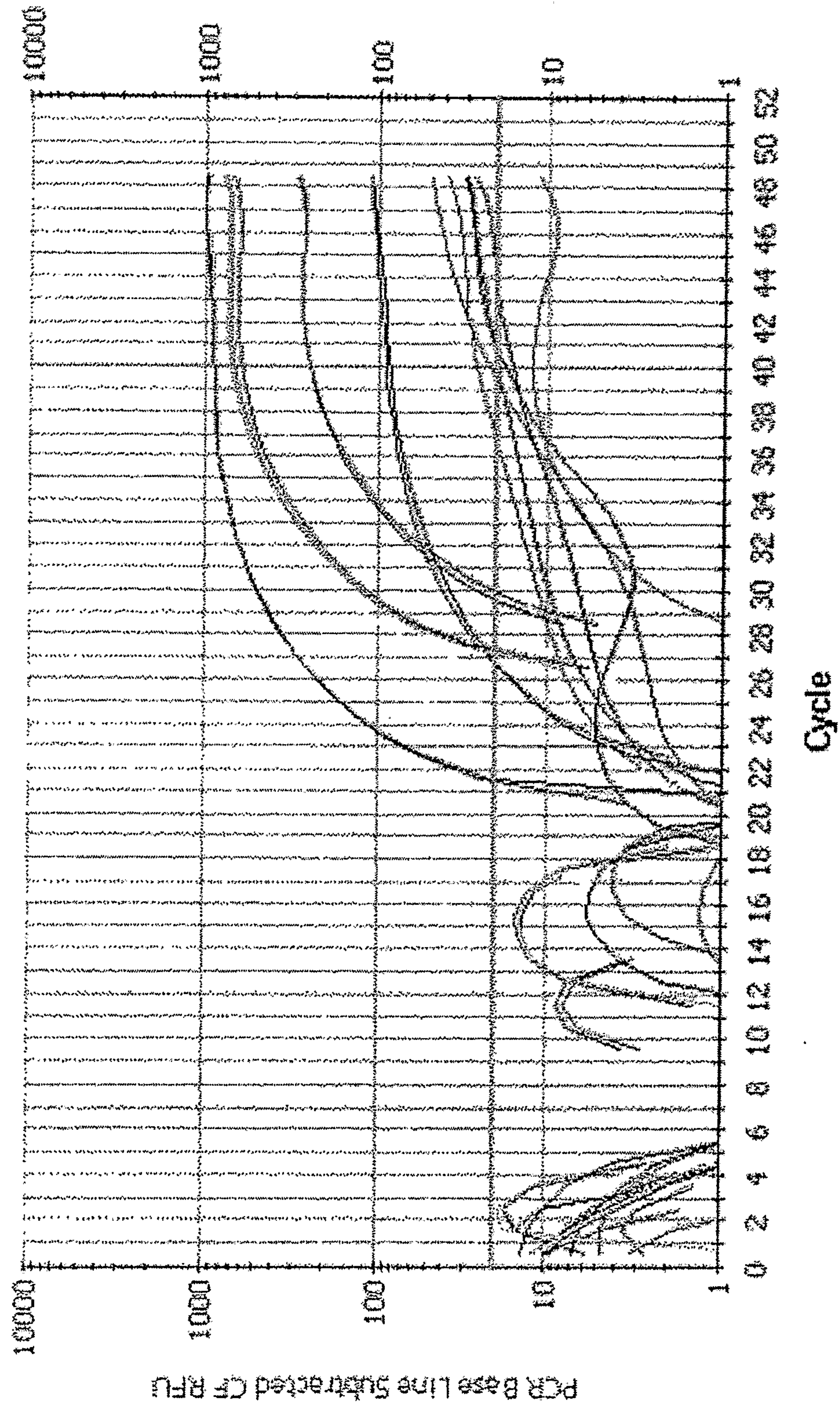
2/25

**Figure 1B**

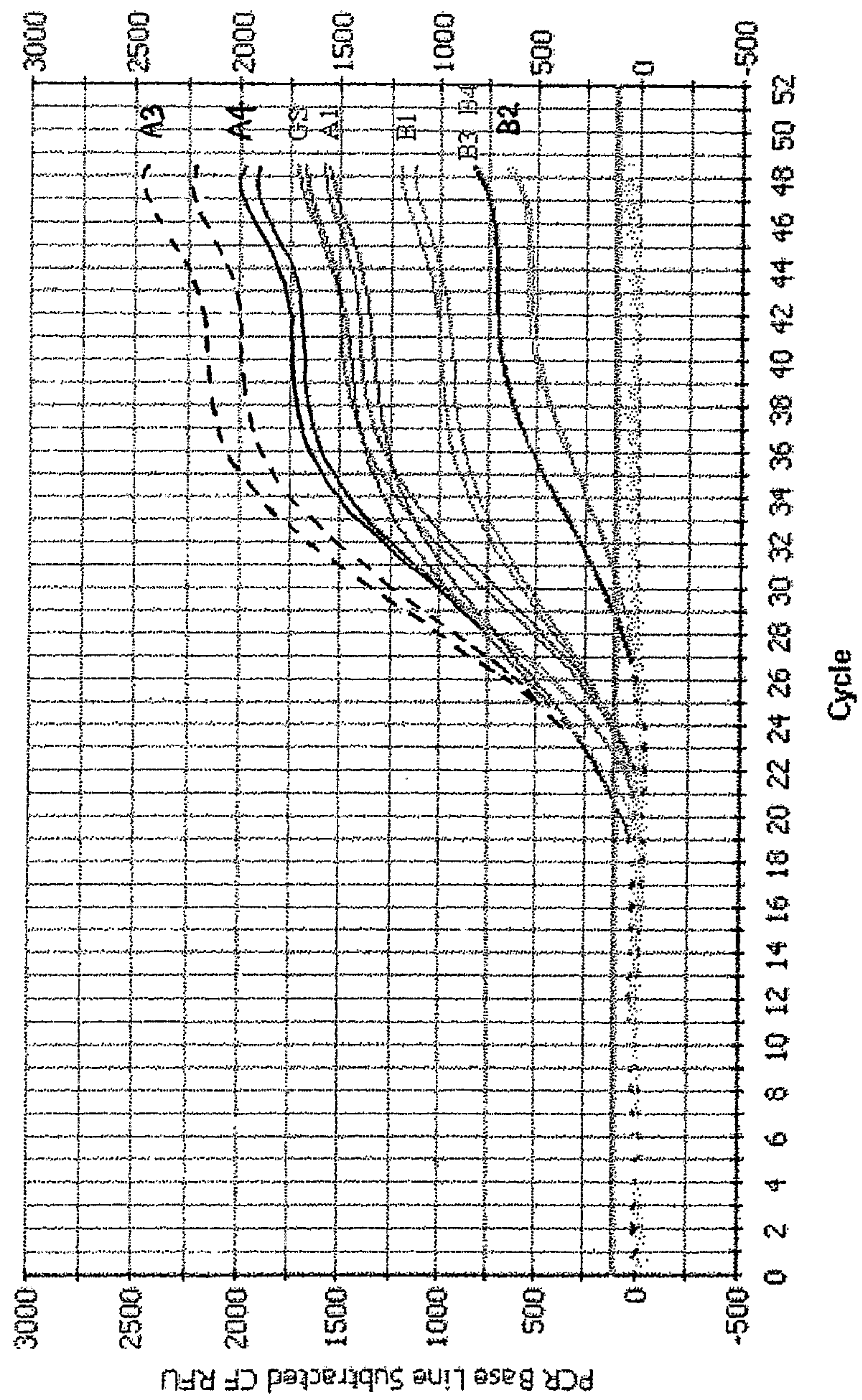
3/25

**Figure 2A**

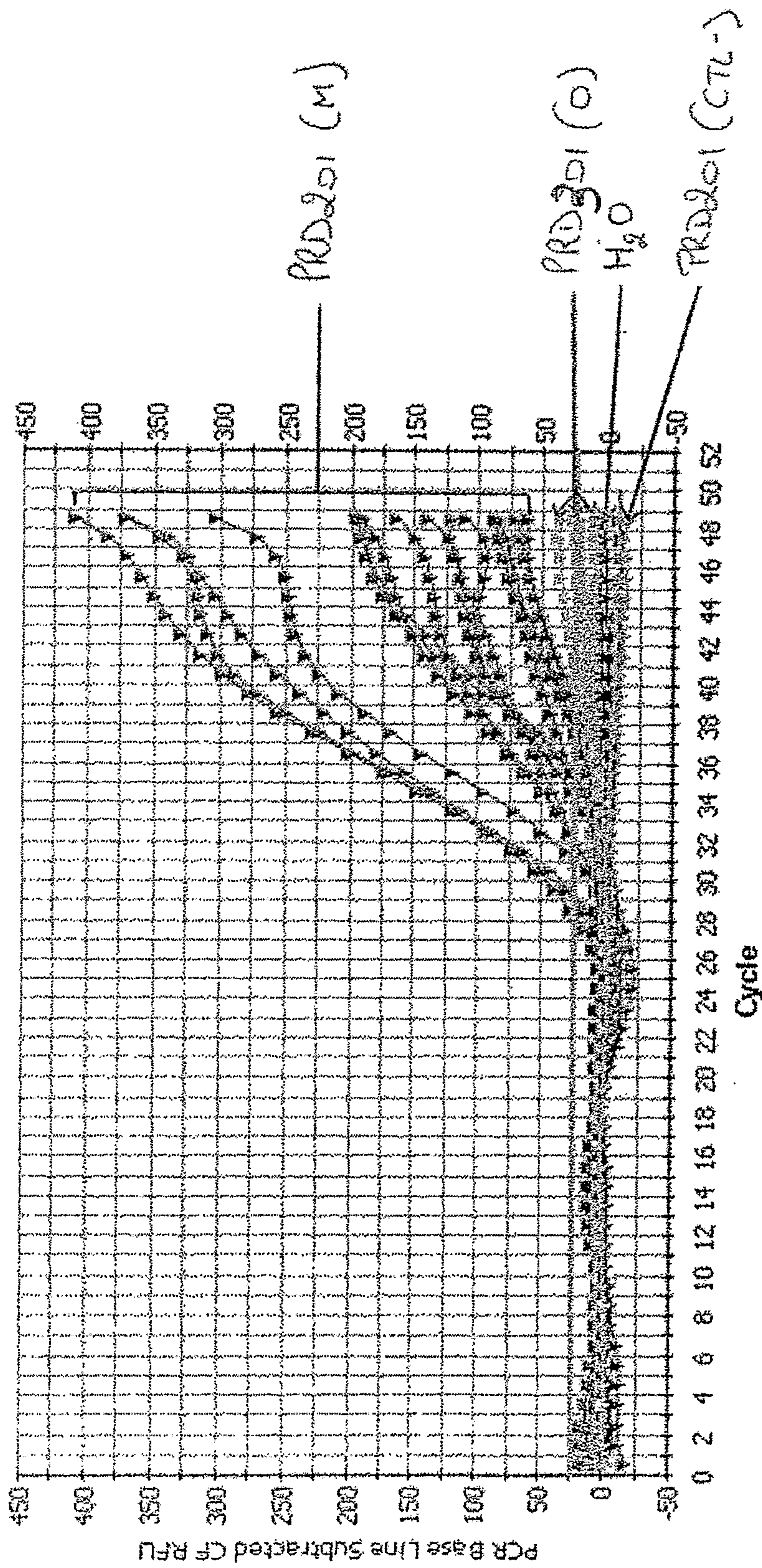
4/25

**Figure 2B**

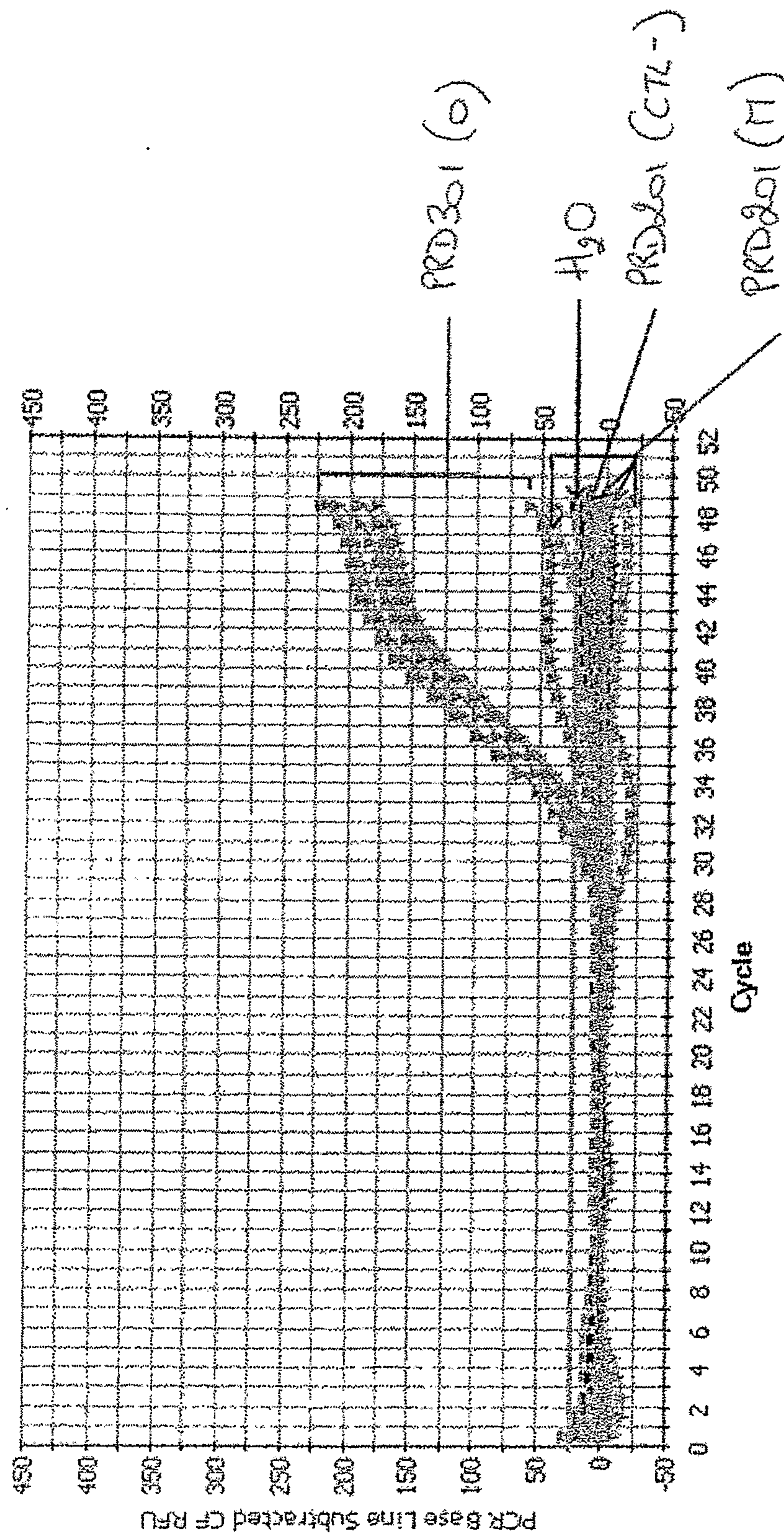
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**Figure 3**

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**Figure 4A**

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**Figure 4B**

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FIGURE 5A : HIV1-M target with 10000 cop, 1000, 100 and 10 cop/PCR and negative controls
FAM DETECTION

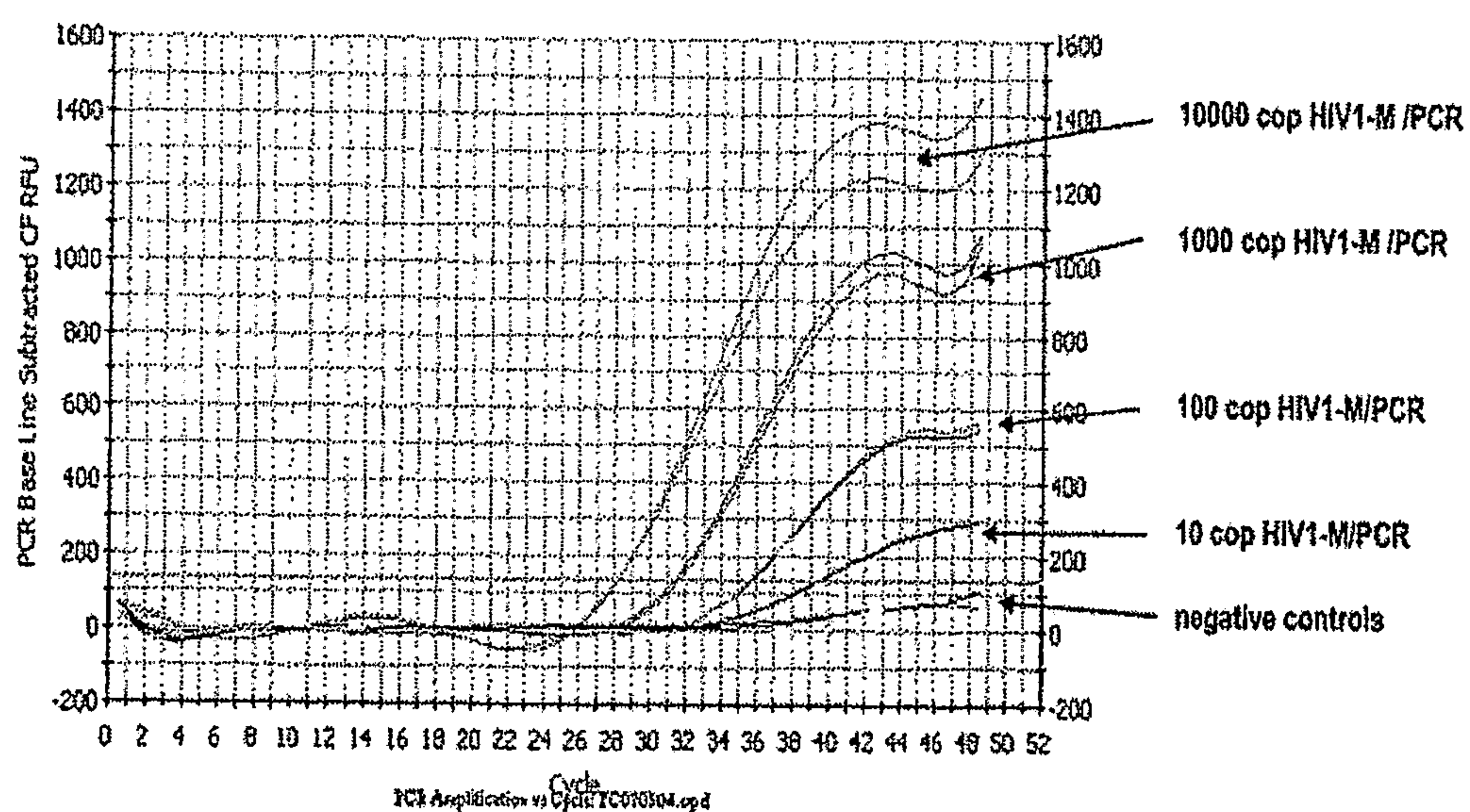
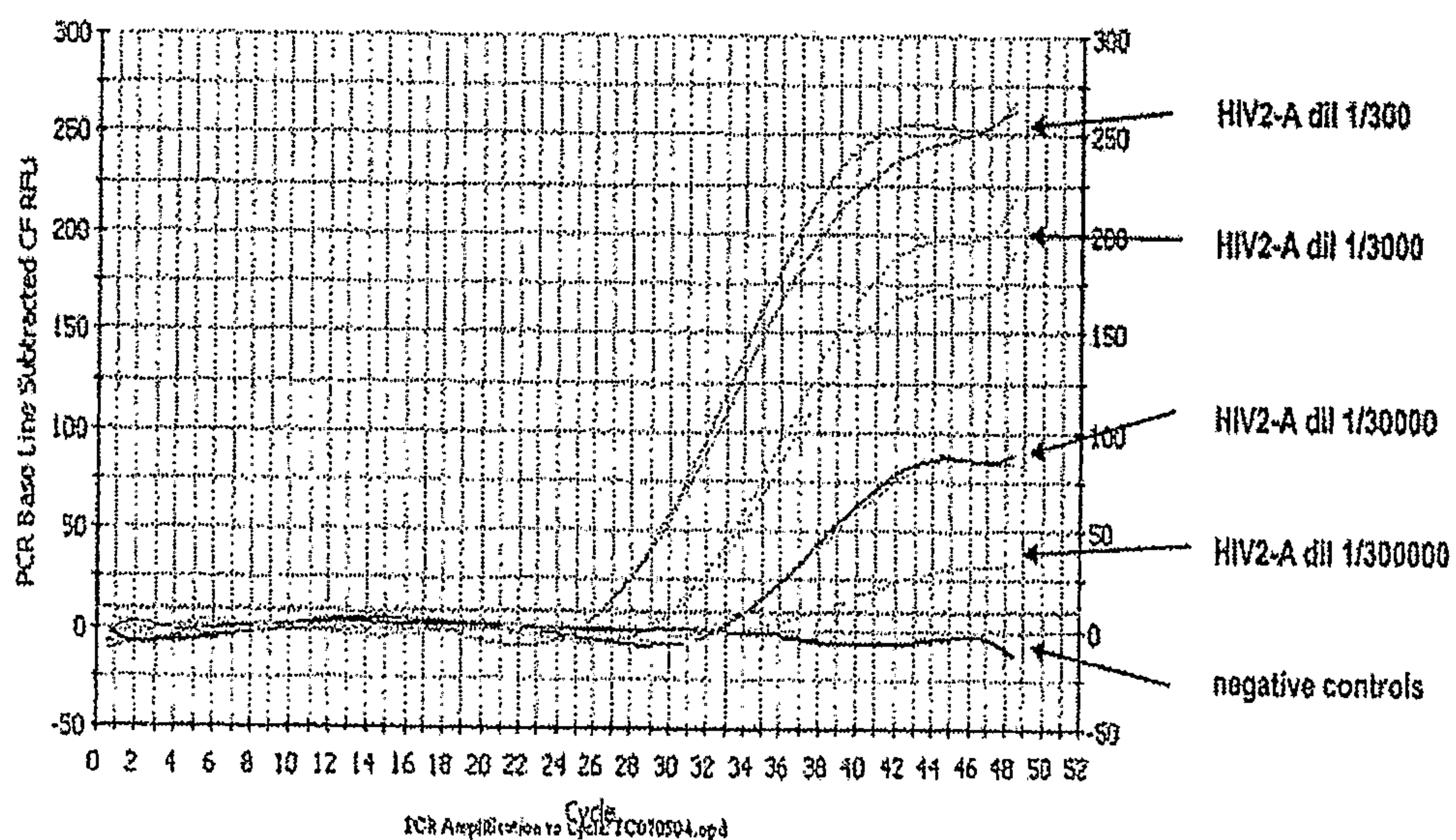


FIGURE 5B : HIV2-A target dil 1/300, 1/3000, 1/30000 and 1/300000 and negative controls
ROX DETECTION



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FIGURE 6A : Experiment a) with HIV2 target dil 1/300, 1/3000, 1/30000
ROX DETECTION

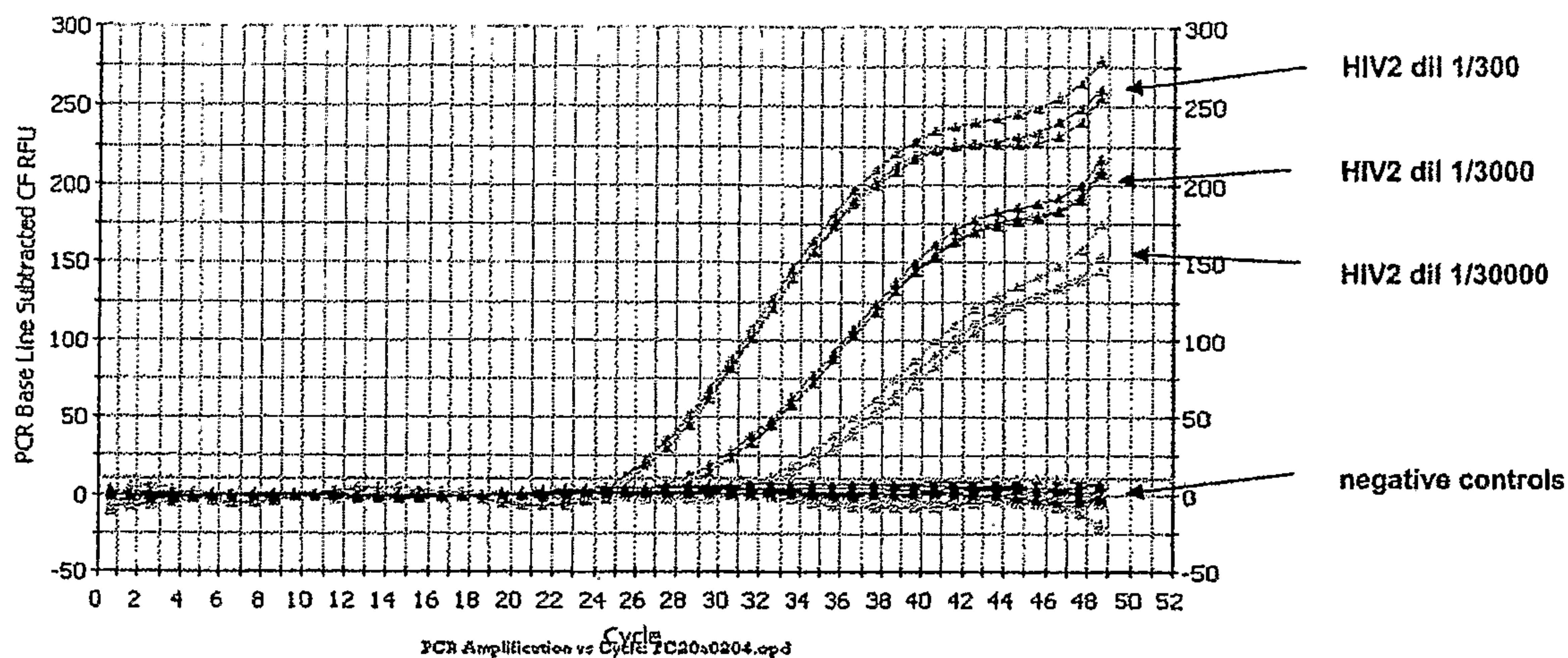


FIGURE 6B : Experiment b) with HIV2 target dil 1/300, 1/3000, 1/30000 + IC 10⁶ cop/PCR
ROX DETECTION

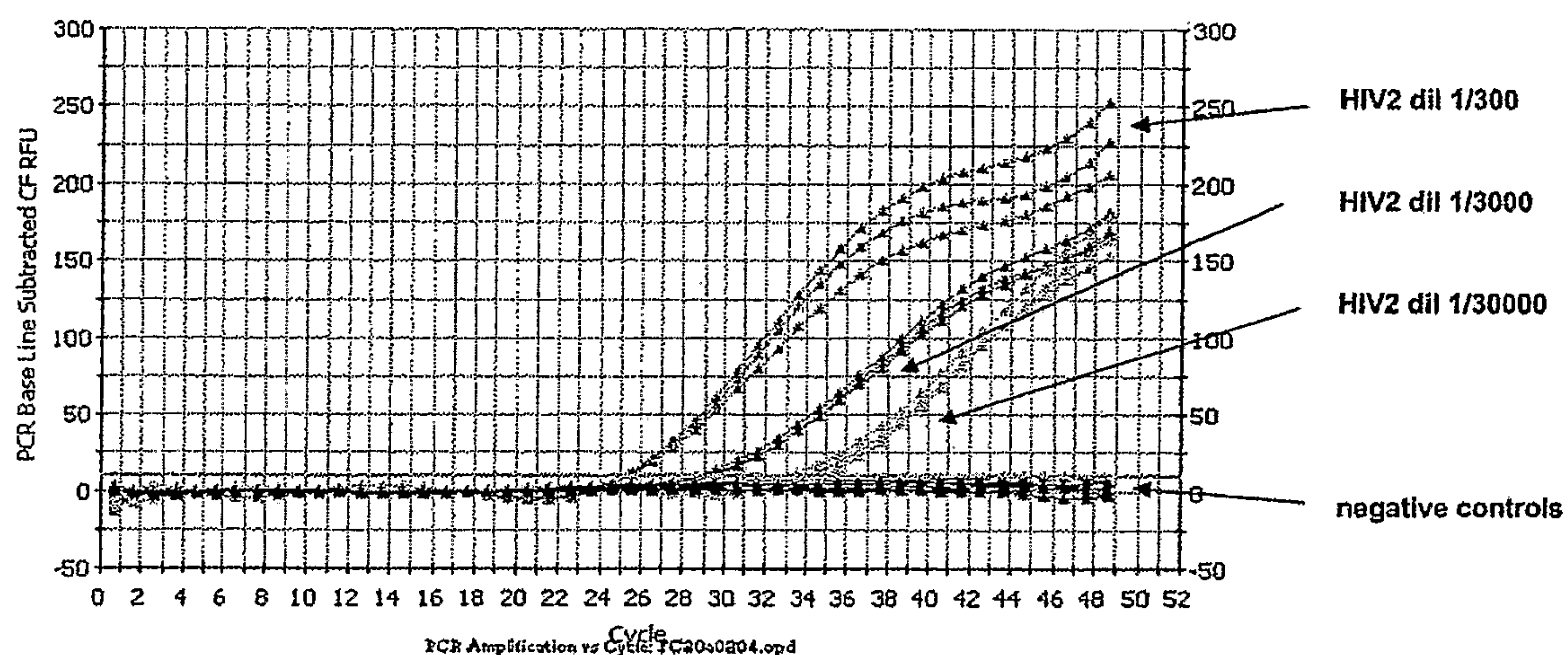
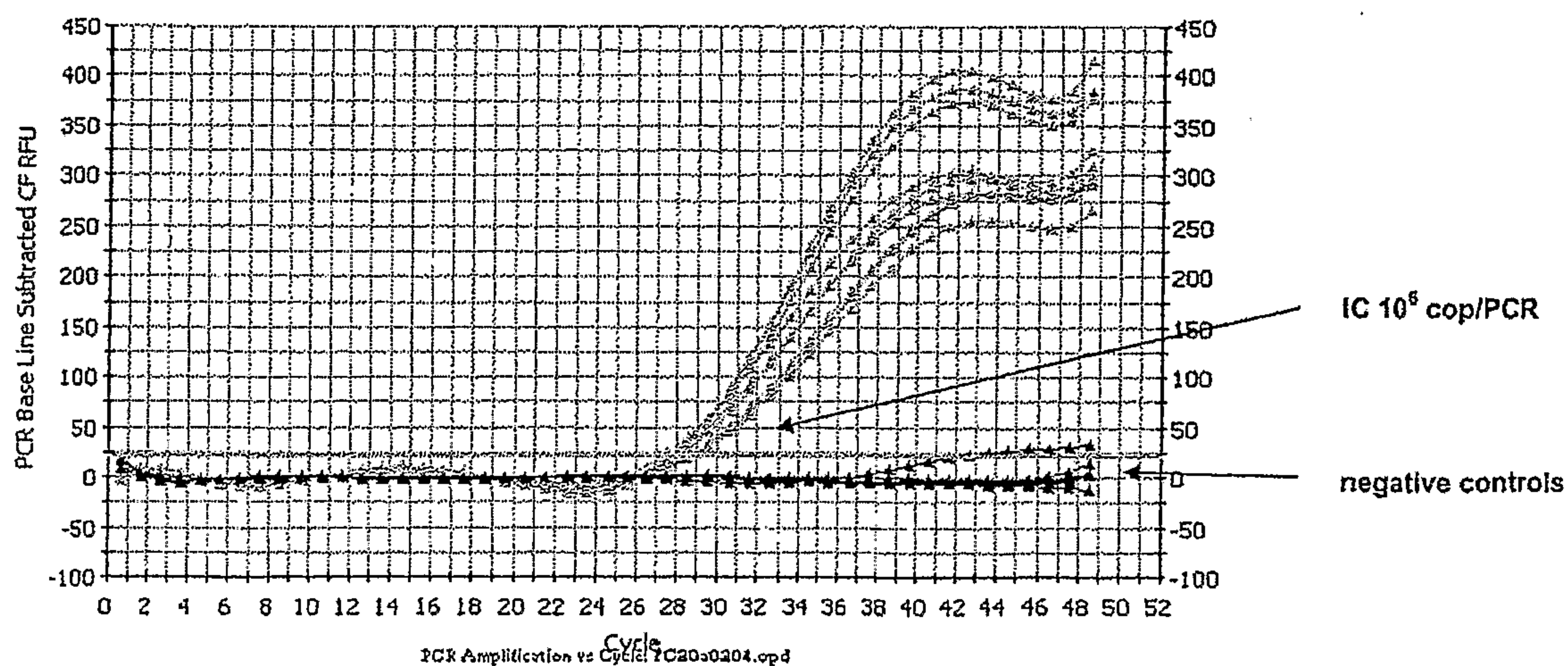


FIGURE 6C : Experiment b) with IC 10⁶ cop/PCR + HIV2 target dil 1/300, 1/3000, 1/30000
TAMRA DETECTION



SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
1		ggagg aaatgaacaa gtagataaat tagtcagtcg tggaaatcagg aaagtactat ttttagatgg aatagataag gcccaagatg aacatgagaa atatacacagt aattggagag caatggcctag tgattttaac ctgccaccctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaaagga gaagccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacacat ttgaa	HIV1-M amplicon		4176 - 4436
2		aattggagag caatggctag tgattttaac ctgccaccctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaaagga gaagccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacacat ttgaa	HIV1-M amplicon		4281 - 4436
3		ggaggaaatg aaaaaataga taaattagta agcaaggata ttagaagagt cctgttccta gaaggaatag accagggcaca agaagatcat gaaaaatc atagtaattg gaaagcacta gctagtgaat ttggactacc accagtggg gccaaggaaa tcattgctag ctgtcctaaa tgtcatataa aaggggaagc aattcatggt caggtagact gcagtcacca agtatggcaa atagattgca cacatatgga a	HIV1-O amplicon		4231 - 4491
4		aattg gaaagcacta gctagtgaat ttggactacc accagtggg gccaaaggaaa tcattgctag ctgtcctaaa tgtcatataa aaggggaagc aattcatggt caggtagact gcagtcacca agtatggcaa atagattgca cacatatgga a	HIV1-O amplicon		4336 - 4491
5		a ggaaggcaga cagcactct cctattaaaa ctggccagta ggtggccaat aacgcacttg cacacagaca atggcccaa cttcacttca caggaagtga agatgggtgc atggtgggta ggtatagAAC aatcctttgg agtacc	HIV2 amplicon		4889 - 5036
305		ggagg aaatgaacaa gtagataaat tagtcagtcg tggaaatcagg aaagtactat ttttagatgg aatagataag gcccaagatg aacatgagaa atatacacagt aattggagag caatggcctag tgattttaac ctgccaccctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaaagga gaagccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacaca	HIV1-M amplicon		4176-4429

FIGURE 7

306	aattggagag caatggcctag tgattttaac ctgccacctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaagga gaagcccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacaca	HIV1-M amplicon	4281-4429
307	ttggagag caatggcctag tgattttaac ctgccacctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaagga gaagcccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacaca	HIV1-M amplicon	4283-4429
308	ttggagag caatggcctag tgattttaac ctgccacctg tagtagcaaa agaaatagta gccagctgtg ataaatgtca gctaaaagga gaagcccatgc atggacaagt agactgtagt ccaggaatat ggcaactaga ttgtacacat t	HIV1-M amplicon	4283-4431
309	ggaggaaatg aaaaaataga taaattagta agcaaggata ttagaagagt cctgttccta gaaggaaatag accagggcaca agaagatcat gaaaaatc atagtaattg gaaagcacta gctagtgaat ttggactacc accagtgggtg gccaaaggaaa tcattgctag ctgtcctaaa tgfcataataa aaggggaagc aattcatggt caggtagact gcagtcaccaga agtatggcaa atagattgca caca	HIV1-O amplicon	4231-4484
310	aattg gaaagcacta gctagtgaat ttggactacc accagtgggtg gccaaaggaaa tcattgctag ctgtcctaaa tgfcataataa aaggggaagc aattcatggt caggtagact gcagtcaccaga agtatggcaa atagattgca caca	HIV1-O amplicon	4336-4484
311	ttg gaaagcacta gctagtgaat ttggactacc accagtgggtg gccaaaggaaa tcattgctag ctgtcctaaa tgfcataataa aaggggaagc aattcatggt caggtagact gcagtcaccaga agtatggcaa atagattgca caca	HIV1-O amplicon	4338-4484

FIGURE 7 (end)

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
6	H1B1f	GGAGGAAATGAACAAGTAGATAAA	HIV1-M forward primer		4176 - 4199
7	H1B4f	TTGGAGAGCAATGGCTAGTGA	HIV1-M forward primer		4283 - 4303
8	H1B25f	TTGGAGAGCAATGGCTAATGA	HIV1-M forward primer		4283 - 4303
9		TTGGAGAGCAATGGCTARTGA	HIV1-M forward primer	d	4283 - 4303
10	H1B11f	<u>AA</u> TTGGAGAGCAATGGCTAGTGA	HIV1-M forward primer	e	4281 - 4303
11	H1B13f	<u>AA</u> TTGGAGAGCAATGGCTAATGA	HIV1-M forward primer	e	4281 - 4303
12		<u>AA</u> TTGGAGAGCAATGGCTARTGA	HIV1-M forward primer	d	4281 - 4303
13	H1B14f	<u>AA</u> TTGGAGAGCAATGGCTAITGA	HIV1-M forward primer	e, i	4281 - 4303
14	H1B12f	<u>TC</u> ACTAATTGGAGAGCAATGGCTAGTGA	HIV1-M forward primer	e, i	4281 - 4303
15		<u>TC</u> ATTAAATTGGAGAGCAATGGCTAATGA	HIV1-M forward primer	e, i	4281 - 4303

FIGURE 8

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
16	H1B10r	TGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer		4429 - 4408
17	H1B32r	TGTGTACAATCTAATTGCCATA	HIV1-M reverse primer		4429 - 4408
18	H1B33r	TGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer		4429 - 4408
19		TGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer		4429 - 4408
20		TGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d	4429 - 4408
21		TGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d	4429 - 4408
22		TGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d	4429 - 4408
23		TGTGTACAATCTARTTGCCATA	HIV1-M reverse primer	d	4429 - 4408
24		TGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d	4429 - 4408
25	H1B16r	AAATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	e	4431 - 4408
26	H1B19r	TTCTAAATGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer	e	4436 - 4408
27	H1B20r	TTCTAAATGTGTACAATCTAATTGCCATA	HIV1-M reverse primer	e	4436 - 4408
28		TTCTAAATGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer	e	4436 - 4408
29		TTCTAAATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	e	4436 - 4408
30		TTCTAAATGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
31		TTCTAAATGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
32		TTCTAAATGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
33		TTCTAAATGTGTACAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
34		TTCTAAATGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
35		TTCTAGATGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer	e	4436 - 4408
36		TTCTAGATGTGTACAATCTAATTGCCATA	HIV1-M reverse primer	e	4436 - 4408
37		TTCTAGATGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer	e	4436 - 4408
38	H1B22r	TTCTAGATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	e	4436 - 4408
39		TTCTAGATGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
40		TTCTAGATGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
41		TTCTAGATGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
42		TTCTAGATGTGTACAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408
43		TTCTAGATGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d, e	4436 - 4408

FIGURE 9

44		<u>TTCTARATGTGTACAATCTAGTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
45		<u>TTCTARATGTGTACAATCTAATTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
46		<u>TTCTARATGTGTGCAATCTAATTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
47		<u>TTCTARATGTGTGCAATCTAGTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
48		<u>TTCTARATGTGTRCAATCTAGTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
49		<u>TTCTARATGTGTGCAATCTARTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
50		<u>TTCTARATGTGTRCAATCTAATTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
51		<u>TTCTARATGTGTACAATCTARTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
52		<u>TTCTARATGTGTRCAATCTARTTGCCATA</u>	HIV1-M reverse primer	d, e	4436 - 4408
53	534	TGTGTICAATCTAATTGCCATA	HIV1-M reverse primer	i	4429 - 4408
54	535	AAATGTGTICAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4431 - 4408
55	H1B23r	TTCTAATGTGTICAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
56	H1B21r	<u>TATGGC</u> TTCTAAATGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
57		<u>TATGGC</u> TTCTAAATGTGTACAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
58		<u>TATGGC</u> TTCTAAATGTGTACAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
59		<u>TATGGC</u> TTCTAAATGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
60		<u>TATGGC</u> TTCTAAATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
61		<u>TATGGC</u> TTCTAAATGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
62		<u>TATGGC</u> TTCTAAATGTGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
63		<u>TATGGC</u> TTCTAAATGTGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
64		<u>TATGGC</u> TTCTAAATGTGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
65		<u>TATGGC</u> TTCTAGATGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
66		<u>TATGGC</u> TTCTAGATGTGTACAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
67		<u>TATGGC</u> TTCTAGATGTGTACAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
68		<u>TATGGC</u> TTCTAGATGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
69		<u>TATGGC</u> TTCTAGATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	e, i	4436 - 4408
70		<u>TATGGC</u> TTCTAGATGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
71		<u>TATGGC</u> TTCTAGATGTGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
72		<u>TATGGC</u> TTCTAGATGTGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
73		<u>TATGGC</u> TTCTAGATGTGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408
74		<u>TATGGC</u> TTCTARATGTGTACAATCTAGTTGCCATA	HIV1-M reverse primer	d, e, i	4436 - 4408

FIGURE 10

75		<u>TATGGC</u> TTCTARATGTGTACAAATCTAATTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
76		<u>TATGGC</u> TTCTARATGTGTACAAATCTARTTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
77		<u>TATGGC</u> TTCTARATGTGTGCAATCTAATTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
78		<u>TATGGC</u> TTCTARATGTGTGCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
79		<u>TATGGC</u> TTCTARATGTGTGCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
80		<u>TATGGC</u> TTCTARATGTGTRCAATCTAGTTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
81		<u>TATGGC</u> TTCTARATGTGTRCAATCTAATTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408
82		<u>TATGGC</u> TTCTARATGTGTRCAATCTARTTGCCATA	HIV1-M reverse primer	d, e, l	4436 - 4408

FIGURE 11

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
83		AAAGAAATAGTAGCCAGCTGTGATAAATGTC	HIV1-M probe	e	4329 - 4359
84		AAAGAAATAGTGGCCAGCTGTGATAAATGTC	HIV1-M probe	e	4329 - 4359
85		AAAGAAATAGTGGCTAGCTGTGATAAATGTC	HIV1-M probe	e	4329 - 4359
86		AAAGAAATAGTAGCTAGCTGTGATAAATGTC	HIV1-M probe	e	4329 - 4359
87		AAAGAAATAGTAGCYAGCTGTGATAAATGTC	HIV1-M probe	e	4329 - 4359
88		AAAGAAATAGTRGCCAGCTGTGATAAATGTC	HIV1-M probe	d, e	4329 - 4359
89		AAAGAAATAGTRGCTAGCTGTGATAAATGTC	HIV1-M probe	d, e	4329 - 4359
90		AAAGAAATAGTGGCYAGCTGTGATAAATGTC	HIV1-M probe	d, e	4329 - 4359
91		AAAGAAATAGTRGCTAGCTGTGATAAATGTC	HIV1-M probe	d, e	4329 - 4359
92	SH1BM4	TGCGCAAGAAATAGTAGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	e, b	4329 - 4359
93		TGCGCAAGAAATAGTGGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	e, b	4329 - 4359
94		TGCGCAAGAAATAGTGGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	e, b	4329 - 4359
95		TGCGCAAGAAATAGTAGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	e, b	4329 - 4359
96		TGCGCAAGAAATAGTAGCYAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, e, b	4329 - 4359
97		TGCGCAAGAAATAGTRGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, e, b	4329 - 4359
98		TGCGCAAGAAATAGTRGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, e, b	4329 - 4359
99		TGCGCAAGAAATAGTGGCYAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, e, b	4329 - 4359
100		TGCGCAAGAAATAGTRGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	e, b	4329 - 4359
101	SH1BM12	CGCGCAAGAAATAGTAGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	e, b	4329 - 4359
102		CGCGCAAGAAATAGTGGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	e, b	4329 - 4359
103		CGCGCAAGAAATAGTGGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	e, b	4329 - 4359
104		CGCGCAAGAAATAGTAGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	e, b	4329 - 4359
105		CGCGCAAGAAATAGTRGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, e, b	4329 - 4359
106		CGCGCAAGAAATAGTRGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, e, b	4329 - 4359
107		CGCGCAAGAAATAGTGGCYAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, e, b	4329 - 4359
108		CGCGCAAGAAATAGTRGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, e, b	4329 - 4359
109		CGCGCAAGAAATAGTRGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, e, b	4329 - 4359

FIGURE 12

110		ATAGTAGCCAGCTGTGATAAATGTC	HIV1-M probe		4335 - 4359
111		ATAGTAGCTAGCTGTGATAAATGTC	HIV1-M probe		4336 - 4359
112		ATAGTGGCCAGCTGTGATAAATGTC	HIV1-M probe		4335 - 4359
113		ATAGTGGCTAGCTGTGATAAATGTC	HIV1-M probe		4335 - 4359
114		ATAGTAGCYAGCTGTGATAAATGTC	HIV1-M probe	d	4335 - 4359
115		ATAGTRGCCAGCTGTGATAAATGTC	HIV1-M probe	d	4336 - 4359
116		ATAGTRGCTAGCTGTGATAAATGTC	HIV1-M probe	d	4335 - 4359
117		ATAGTGGCYAGCTGTGATAAATGTC	HIV1-M probe	d	4335 - 4359
118		ATAGTRGCYAGCTGTGATAAATGTC	HIV1-M probe	d	4335 - 4359
119	SH1BM5	TGCGCATAGTAGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	b	4335 - 4359
120		TGCGCATAGTAGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	b	4335 - 4359
121		TGCGCATAGTGGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	b	4335 - 4359
122		TGCGCATAGTGGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	b	4335 - 4359
123		TGCGCATAGTAGCYAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, b	4335 - 4359
124		TGCGCATAGTRGCCAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, b	4335 - 4359
125		TGCGCATAGTRGCTAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, b	4335 - 4359
126		TGCGCATAGTGGCYAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, b	4335 - 4359
127		TGCGCATAGTRGCYAGCTGTGATAAATGTCGCGCA	HIV1-M probe	d, b	4335 - 4359
128	SH1BM10	CGCGCATAGTAGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	b	4335 - 4359
129	SH1BM13	CGCGCATAGTAGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	b	4335 - 4359
130	SH1BM14	CGCGCATAGTGGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	b	4335 - 4359
131		CGCGCATAGTGGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	b	4335 - 4359
132		CGCGCATAGTAGCYAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, b	4335 - 4359
133		CGCGCATAGTRGCCAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, b	4335 - 4359
134		CGCGCATAGTRGCTAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, b	4335 - 4359
135		CGCGCATAGTGGCYAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, b	4335 - 4359
136		CGCGCATAGTRGCYAGCTGTGATAAATGTCGCGCG	HIV1-M probe	d, b	4335 - 4359
137		ATAGTIGCIAGCTGTGATAAATGTC	HIV1-M probe	i	4335 - 4359
138	SH1BM15	CGCGCATAGTIGCIAGCTGTGATAAATGTCGCGCG	HIV1-M probe	i, b	4335 - 4359

FIGURE 13

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
139	H1B3f	GGAGGAAATGAAAAATAGATAAA	HIV1-O forward primer		4231 - 4254
140	H1B5f	TTGGAGAGCACTAGCTAGTGA	HIV1-O forward primer		4338 - 4358
141		TTGGAGAGCATTAGCTAGTGA	HIV1-O forward primer		4338 - 4358
142		TTGGAAAGCACTAGCTAGTGA	HIV1-O forward primer		4338 - 4358
143		TTGGAAAGCATTAGCTAGTGA	HIV1-O forward primer		4338 - 4358
144		TTGGAGAGCAYTAGCTAGTGA	HIV1-O forward primer	d	4338 - 4358
145		TTGGARAGCACTAGCTAGTGA	HIV1-O forward primer	d	4338 - 4358
146		TTGGAAAGCAYTAGCTAGTGA	HIV1-O forward primer	d	4338 - 4358
147		TTGGARAGCATTAGCTAGTGA	HIV1-O forward primer	d	4338 - 4358
148		TTGGARAGCAYTAGCTAGTGA	HIV1-O forward primer	d	4338 - 4358
149	H1B15f	<u>AA</u> TTGGAGAGCATTAGCTAGTGA	HIV1-O forward primer	e	4336 - 4358
150	H1B16f	<u>AA</u> TTGGAAAGCACTAGCTAGTGA	HIV1-O forward primer	e	4336 - 4358
151	H1B17f	<u>AA</u> TTGGAAAGCATTAGCTAGTGA	HIV1-O forward primer	e	4336 - 4358
152		<u>AA</u> TTGGAGAGCACTAGCTAGTGA	HIV1-O forward primer	e	4336 - 4358
153		<u>AA</u> TTGGARAGCACTAGCTAGTGA	HIV1-O forward primer	d, e	4336 - 4358
154		<u>AA</u> TTGGAAAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e	4336 - 4358
155		<u>AA</u> TTGGARAGCATTAGCTAGTGA	HIV1-O forward primer	d, e	4336 - 4358
156		<u>AA</u> TTGGARAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e	4336 - 4358
157		<u>AA</u> TTGGARAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e	4336 - 4358
158	H1B18f	<u>AA</u> TTGGAAGCAITAGCTAGTGA	HIV1-O forward primer	e, i	4336 - 4358
159		<u>TC</u> ACTAAATTGGAGAGCATTAGCTAGTGA	HIV1-O forward primer	e, l	4336 - 4358
160		<u>TC</u> ACTAAATTGGAAAGCACTAGCTAGTGA	HIV1-O forward primer	e, l	4336 - 4358
161		<u>TC</u> ACTAAATTGGAAAGCATTAGCTAGTGA	HIV1-O forward primer	e, l	4336 - 4358
162		<u>TC</u> ACTAAATTGGAGAGCACTAGCTAGTGA	HIV1-O forward primer	e, l	4336 - 4358
163		<u>TC</u> ACTAAATTGGARAGCACTAGCTAGTGA	HIV1-O forward primer	d, e, l	4336 - 4358
164		<u>TC</u> ACTAAATTGGAAAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e, l	4336 - 4358
165		<u>TC</u> ACTAAATTGGARAGCATTAGCTAGTGA	HIV1-O forward primer	d, e, l	4336 - 4358
166		<u>TC</u> ACTAAATTGGARAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e, l	4336 - 4358
167		<u>TC</u> ACTAAATTGGARAGCAYTAGCTAGTGA	HIV1-O forward primer	d, e, l	4336 - 4358

FIGURE 14

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
168	H1B13r	TGTGTGCAATCTATTTGCCATA	HIV1-O reverse primer		4484 - 4463
169		TGTGTACAATCTATTTGCCATA	HIV1-O reverse primer		4484 - 4463
170		TGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer		4484 - 4463
171	H1B24r	<u>TTCTAAAT</u> GTGTACAATCTATTTGCCATA	HIV1-O reverse primer	e	4491 - 4463
172	H1B25r	<u>TTCTAGAT</u> GTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	e	4491 - 4463
173		<u>TTCTAAAT</u> GTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	e	4491 - 4463
174		<u>TTCTAGAT</u> GTGTACAATCTATTTGCCATA	HIV1-O reverse primer	e	4491 - 4463
175		<u>TTCTARAT</u> GTGTACAATCTATTTGCCATA	HIV1-O reverse primer	d, e	4491 - 4463
176		<u>TTCTARAT</u> GTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	d, e	4491 - 4463
177		<u>TTCTAAAT</u> GTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	d, e	4491 - 4463
178		<u>TTCTAGAT</u> GTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	d, e	4491 - 4463
179		<u>TTCTARAT</u> GTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	e	4491 - 4463
180	H1B26r	<u>TTCTAIA</u> TGTGTICAATCTATTTGCCATA	HIV1-O reverse primer	e, i	4491 - 4463
181		<u>TATGGC</u> TTCTAAATGTGTACAATCTATTTGCCATA	HIV1-O reverse primer	e, l	4491 - 4463
182		<u>TATGGC</u> TTCTAGATGTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	e, l	4491 - 4463
183		<u>TATGGC</u> TTCTAAATGTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	e, l	4491 - 4463
184		<u>TATGGC</u> TTCTAGATGTGTACAATCTATTTGCCATA	HIV1-O reverse primer	e, l	4491 - 4463
185		<u>TATGGC</u> TTCTARATGTGTACAATCTATTTGCCATA	HIV1-O reverse primer	d, e, l	4491 - 4463
186		<u>TATGGC</u> TTCTARATGTGTGCAATCTATTTGCCATA	HIV1-O reverse primer	d, e, l	4491 - 4463
187		<u>TATGGC</u> TTCTAAATGTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	d, e, l	4491 - 4463
188		<u>TATGGC</u> TTCTAGATGTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	d, e, l	4491 - 4463
189		<u>TATGGC</u> TTCTARATGTGTGTRCAATCTATTTGCCATA	HIV1-O reverse primer	d, e, l	4491 - 4463

FIGURE 15

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
190		GAAATCATTTGCTAGTTGTCCTAAATGTCATAT	HIV1-O probe		4387 - 4417
191	SH1B02	TGCGCGGAAATCATTTGCTAGTTGTCCTAAATGTCATATGCGCA	HIV1-O probe	b	4387 - 4417
192	SH1B07	CGCGCGGAAATCATTTGCTAGTTGTCCTAAATGTCATATGCGCG	HIV1-O probe	b	4387 - 4417
193		AGTCTACCTGACCATGAATTGCTTCCCCCTTTTA	HIV1-O probe		4449 - 4417
194	SH1B010	CGCGCAAGTCTACCTGACCATGAATTGCTTCCCCCTTTTATGCGCG	HIV1-O probe	b	4449 - 4417

FIGURE 16

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
195	H2A3f	AGGAAGRCARACAGCACTCTTC	HIV2 forward primer	d	4889 - 4913
196	H2A9f	AGGAAGGCAAAACAGCACTCTTC	HIV2 forward primer	c	4889 - 4913
197		AGGAAGACARACAGCACTCTTC	HIV2 forward primer	d	4889 - 4913
198		AGGAAGGCARACAGCACTCTTC	HIV2 forward primer	d	4889 - 4913
199		AGGAAGACAAACAGCACTCTTC	HIV2 forward primer		4889 - 4913
200		AGGAAGGCAGACAGCACTCTTC	HIV2 forward primer		4889 - 4913
201		AGGAAGACAGACAGCACTCTTC	HIV2 forward primer		4889 - 4913
202		AGGAAGRCAAAACAGCACTCTTC	HIV2 forward primer	d	4889 - 4913
203		AGGAAGRCAGACAGCACTCTTC	HIV2 forward primer	d	4889 - 4913
204	H2A10f	AGGAAGACAGACAGCTCTCTTC	HIV2 forward primer	c	4889 - 4913
205		AGGAAGRCARACAGCTCTCTTC	HIV2 forward primer	d	4889 - 4913
206		AGGAAGACAAACAGCTCTCTTC	HIV2 forward primer		4889 - 4913
207		AGGAAGGCAGACAGCTCTCTTC	HIV2 forward primer		4889 - 4913
208		AGGAAGGCAAAACAGCTCTCTTC	HIV2 forward primer		4889 - 4913
209		AGGAAGRCAGACAGCTCTCTTC	HIV2 forward primer	d	4889 - 4913
210		AGGAAGRCAAAACAGCTCTCTTC	HIV2 forward primer	d	4889 - 4913
211		AGGAAGACARACAGCTCTCTTC	HIV2 forward primer	d	4889 - 4913
212		AGGAAGGCARACAGCTCTCTTC	HIV2 forward primer	d	4889 - 4913
213		AGGAAGACAAACAGCWCTCTTC	HIV2 forward primer		4889 - 4913
214		AGGAAGGCAGACAGCWCTCTTC	HIV2 forward primer		4889 - 4913
215		AGGAAGACAGACAGCWCTCTTC	HIV2 forward primer		4889 - 4913
216		AGGAAGGCAAAACAGCWCTCTTC	HIV2 forward primer		4889 - 4913
217		AGGAAGRCAGACAGCWCTCTTC	HIV2 forward primer	d	4889 - 4913
218		AGGAAGRCAAAACAGCWCTCTTC	HIV2 forward primer	d	4889 - 4913
219		AGGAAGACARACAGCWCTCTTC	HIV2 forward primer	d	4889 - 4913
220		AGGAAGGCARACAGCWCTCTTC	HIV2 forward primer	d	4889 - 4913
221		AGGAAGRCARACAGCWCTCTTC	HIV2 forward primer	d	4889 - 4913
222	539	CAGGAAGICAACAGCCTCTTCCT	HIV2 forward primer	e, i	4888 - 4915

FIGURE 17

223	H2A7f	GAAGAGAGGAAGRCARACAGCACTCTTC	HIV2 forward primer	d, l	4889 - 4913
224		GAAGAGAGGAAGACAAACAGCACTCTTC	HIV2 forward primer	l	4889 - 4913
225		GAAGAGAGGAAGGCAGACAGCACTCTTC	HIV2 forward primer	l	4889 - 4913
226		GAAGAGAGGAAGGCCAAACAGCACTCTTC	HIV2 forward primer	l	4889 - 4913
227		GAAGAGAGGAAGACAGACAGCACTCTTC	HIV2 forward primer	l	4889 - 4913
228		GAAGAGAGGAAGACARACAGCACTCTTC	HIV2 forward primer	d, l	4889 - 4913
229		GAAGAGAGGAAGGCARACAGCACTCTTC	HIV2 forward primer	d, l	4889 - 4913
230		GAAGAGAGGAAGRCAAACAGCACTCTTC	HIV2 forward primer	d, l	4889 - 4913
231		GAAGAGAGGAAGRCAGACAGCACTCTTC	HIV2 forward primer	d, l	4889 - 4913
232		GAAGAGAGGAAGRCARACAGCTCTCTTC	HIV2 forward primer	d, l	4889 - 4913
233		GAAGAGAGGAAGACAAACAGCTCTCTTC	HIV2 forward primer	l	4889 - 4913
234		GAAGAGAGGAAGGCAGACAGCTCTCTTC	HIV2 forward primer	l	4889 - 4913
235		GAAGAGAGGAAGGCCAAACAGCTCTCTTC	HIV2 forward primer	l	4889 - 4913
236		GAAGAGAGGAAGACAGACAGCTCTCTTC	HIV2 forward primer	l	4889 - 4913
237		GAAGAGAGGAAGACARACAGCTCTCTTC	HIV2 forward primer	d, l	4889 - 4913
238		GAAGAGAGGAAGGCARACAGCTCTCTTC	HIV2 forward primer	d, l	4889 - 4913
239		GAAGAGAGGAAGRCAAACAGCTCTCTTC	HIV2 forward primer	d, l	4889 - 4913
240		GAAGAGAGGAAGRCAGACAGCTCTCTTC	HIV2 forward primer	d, l	4889 - 4913
241		GAAGAGAGGAAGACAAACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
242		GAAGAGAGGAAGGCAGACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
243		GAAGAGAGGAAGGCCAAACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
244		GAAGAGAGGAAGACAGACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
245		GAAGAGAGGAAGACARACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
246		GAAGAGAGGAAGGCARACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
247		GAAGAGAGGAAGRCAAACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
248		GAAGAGAGGAAGRCAGACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913
249		GAAGAGAGGAAGRCARACAGCWCTCTTC	HIV2 forward primer	d, l	4889 - 4913

FIGURE 18

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
250	H2A3r	GGTACTCCRAAGGTTTGTTCTAT	HIV2 reverse primer	d	5036 - 5014
251		GGTACTCCRAAGGATTGTTCTAT	HIV2 reverse primer	d	5036 - 5014
252		GGTACTCCRAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
253		GGTACTCCRAAGGATTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
254		GGTACTCCRAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
255		GGTACTCCRAAGGTTTGTTCTAT	HIV2 reverse primer	d	5036 - 5014
256		GGTACTCCRAAGGATTGYTCTAT	HIV2 reverse primer	d	5036 - 5014
257		GGTACTCCRAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
258	H2A8r	GGTACTCCAAAGGATTGCTCTAT	HIV2 reverse primer	c	5036 - 5014
259		GGTACTCCAAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
260		GGTACTCCAAAGGTTTGTTCTAT	HIV2 reverse primer	d	5036 - 5014
261		GGTACTCCAAAGGTTGYTCTAT	HIV2 reverse primer	d	5036 - 5014
262		GGTACTCCAAAGGATTGYTCTAT	HIV2 reverse primer	d	5036 - 5014
263		GGTACTCCAAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
264		GGTACTCCAAAGGTTTGTTCTAT	HIV2 reverse primer		5036 - 5014
265		GGTACTCCAAAGGATTGTTCTAT	HIV2 reverse primer		5036 - 5014
266		GGTACTCCAAAGGTTTGCTCTAT	HIV2 reverse primer		5036 - 5014
267	H2A9r	GGTACTCCGAAGGTTTGTTCTAT	HIV2 reverse primer	c	5036 - 5014
268		GGTACTCCGAAGGATTGTTCTAT	HIV2 reverse primer		5036 - 5014
269		GGTACTCCGAAGGTTTGCTCTAT	HIV2 reverse primer		5036 - 5014
270		GGTACTCCGAAGGATTGCTCTAT	HIV2 reverse primer		5036 - 5014
271		GGTACTCCGAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
272		GGTACTCCGAAGGTTTGTTCTAT	HIV2 reverse primer	d	5036 - 5014
273		GGTACTCCGAAGGTTGYTCTAT	HIV2 reverse primer	d	5036 - 5014
274		GGTACTCCGAAGGATTGYTCTAT	HIV2 reverse primer	d	5036 - 5014
275		GGTACTCCGAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
276		GGTACTCCRAAGGTTTGCTCTAT	HIV2 reverse primer	d	5036 - 5014
277	H2A10r	GGTACTCCIAAGGTTTGITCTAT	HIV2 reverse primer	i	5036 - 5014

FIGURE 19

278	n° 538	GGTACTCC	AAGGTTGTTCTAT	HIV2 reverse primer	i	5036 - 5014
279	H2A7r	ATAGAACGGTACTCCRAAGGTTTGTTCTAT		HIV2 reverse primer	d, l	5036 - 5014
280		ATAGAACGGTACTCCGAAGGTTTGTTCTAT		HIV2 reverse primer	l	5036 - 5014
281		ATAGAACGGTACTCCRAAGGATTGTTCTAT		HIV2 reverse primer	d, l	5036 - 5014
282		ATAGAACGGTACTCCAAAGGTTTGTTCTAT		HIV2 reverse primer	d, l	5036 - 5014
283		ATAGAACGGTACTCCAAAGGTTTGTTCTAT		HIV2 reverse primer	l	5036 - 5014
284		ATAGAACGGTACTCCAAAGGATTGTTCTAT		HIV2 reverse primer	l	5036 - 5014
285		ATAGAACGGTACTCCGAAGGATTGTTCTAT		HIV2 reverse primer	l	5036 - 5014
286		ATAGAACGGTACTCCGAAGGTTTGTTCTAT		HIV2 reverse primer	d, l	5036 - 5014
287		ATAGAACGGTACTCCRAAGGTTTGTTCTAT		HIV2 reverse primer	d, l	5036 - 5014
288		ATAGAGCGGTACTCCRAAGGTTTGCTCTAT		HIV2 reverse primer	d, l	5036 - 5014
289		ATAGAGCGGTACTCCRAAGGATTGCTCTAT		HIV2 reverse primer	d, l	5036 - 5014
290		ATAGAGCGGTACTCCAAAGGATTGCTCTAT		HIV2 reverse primer	l	5036 - 5014
291		ATAGAGCGGTACTCCAAAGGTTGCTCTAT		HIV2 reverse primer	d, l	5036 - 5014
292		ATAGAGCGGTACTCCAAAGGTTTGCTCTAT		HIV2 reverse primer	l	5036 - 5014
293		ATAGAGCGGTACTCCGAAGGTTTGCTCTAT		HIV2 reverse primer	l	5036 - 5014
294		ATAGAGCGGTACTCCGAAGGATTGCTCTAT		HIV2 reverse primer	l	5036 - 5014
295		ATAGAGCGGTACTCCGAAGGTTTGCTCTAT		HIV2 reverse primer	d, l	5036 - 5014
296		ATAGAGCGGTACTCCRAAGGTTTGCTCTAT		HIV2 reverse primer	d, l	5036 - 5014

FIGURE 20

SEQ ID No.	Name	5' to 3' sequence	Preferred function	comment	positions
297		GGCCAAT A ACACACACTTGCACACA	HIV2-A probe		4932 - 4956
298	SH2A9a	AGCGCGGCCCAATAACACACACTTGCACACAGCGCT	HIV2-A probe	b	4932 - 4956
299	SH2A14a	CGCGCGGCCCAATAACACACACTTGCACACAGCGCGG	HIV2-A probe	b	4932 - 4956
300		GCCTATC A CACACACCTGCACACA	HIV2-B probe		4933 - 4956
301	SH2A1b	AGCGCGCCTATC A CACACACCTGCACACAGCGCT	HIV2-B probe	b	4933 - 4956
302	SH2A2b	CGCGCGCCTATC A CACACACCTGCACACAGCGCGG	HIV2-B probe	b	4933 - 4956
303	SH2A3b	CGCGCGCCTATC A CACACACCTGCACACAGCGGG	HIV2-B probe	b	4933 - 4956
304	SH2AB	CGCGCGGCCCA T ACACACACITGCACACAGCGCGG	HIV2 probe	i	4932 - 4956

FIGURE 21