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(71) Applicant: **THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY, DEPARTMENT OF HEALTH AND HUMAN SERVICES** [US/US]; 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892 (US).

(72) Inventors: **BRENCHLEY, Jason Michael**; c/o The United States of America, as Represented by the Secretary, Department of Health and Human Services, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892 (US). **LANGNER, Charlotte Anna**; c/o The United States of America, as Represented by the Secretary, Department of Health and Human Services, 6011 Executive Boulevard, Suite 325, MSC 7660, Bethesda, Maryland 20892 (US).

(74) Agent: **GALANT, Ron** et al.; Polsinelli PC, 105 West Vandalia, Suite 400, Edwardsville, Illinois 62025 (US).

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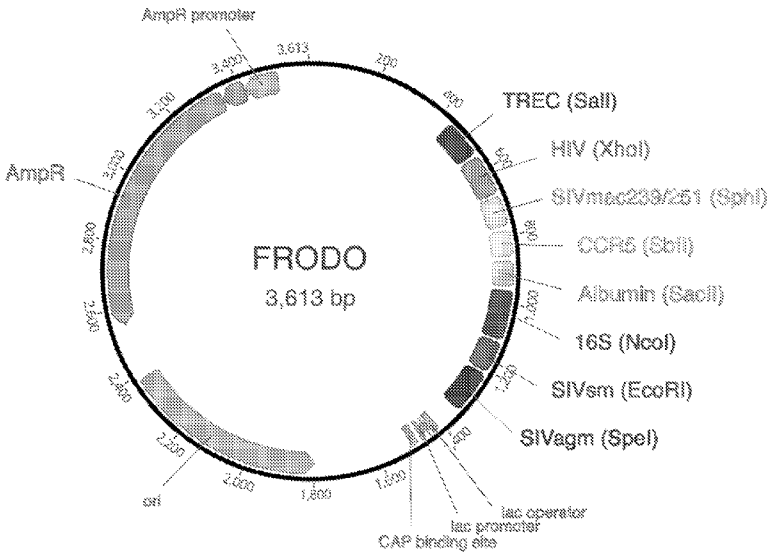
(54) Title: DNA VECTOR AND USES THEREOF FOR DETECTING HIV AND SIV

FIG. 1



(57) Abstract: The present invention relates to a vector and its use as a standard in methods for detecting HIV and SIV.

[Continued on next page]

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DNA VECTOR AND USES THEREOF FOR DETECTING HIV AND SIV

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

[0001] This invention was made with government support under project number ZIA AI001029 by the National Institutes of Health. The United States government has certain rights in the invention.

CROSS REFERENCE OF RELATED APPLICATION

[0002] This application claims the benefit of U.S. Provisional Patent Application Serial No. 63/128,392 filed on December 21, 2021 which is hereby incorporated by reference in its entirety.

FIELD OF THE INVENTION

[0003] Provided herein is a vector and its use as a standard in methods for detecting HIV and SIV.

BACKGROUND OF THE INVENTION

[0004] Quantitative, real-time PCR (RT-qPCR) is a very commonly used technology in immunology, bacteriology, and virology. This technique can be used to enumerate numbers of viruses, bacterial taxa, and immunological genes of interest in biological samples from in vitro and ex vivo experiments. This approach is used to determine the numbers of human immunodeficiency virus (HIV) or simian immunodeficiency virus (SIV) DNA copies per cell of interest after viral infection both in vitro and in vivo (Beaumier et al., 2009; Brenchley et al., 2004; Chomont et al., 2009; Lai et al., 2019; Mattapallil et al., 2005). These experiments involve measuring quantities of viral DNA relative to the number of host cells, determined by copy numbers of targeted host genes, via quantitative PCR (qPCR). Moreover, qPCR is often used to measure thymic output and immunological recovery after immunological insult by measuring the numbers of rearranged T cell receptor excision circles (TREC, generated by rearrangement of T cell receptor genes, resulting in excised, circular, DNA) (D. Douek, 2004; D. C. Douek et al., 2001; McFarland, Douek, Koup, & Picker, 2000; Muraro et al., 2005). Analysis of TREC numbers has also been used to calculate the numbers of cell divisions which occur in vivo among

populations of T cells (Brenchley et al., 2004; Tanaskovic, Fernandez, Price, Lee, & French, 2010). Finally, levels of DNA encoding for bacterial 16S ribosomal RNA are used to determine levels of bacterial DNA present in biological samples (Jiang et al., 2009; Klase et al., 2015).

[0005] To yield absolute quantities of gene copies, serial dilutions of known quantities of these amplicons are simultaneously analyzed with unknown samples and standard curves of cycle threshold versus analyte quantity are created (D. C. Douek et al., 1998). These known levels of amplicons are generally created by ligating amplicons into plasmid DNA followed by transformation of competent bacteria, amplification of the plasmid, and subsequent purification and quantification. Historically, one plasmid contains one amplicon of interest and laboratories which perform multiple qPCR analyses must maintain multiple plasmid standards. This approach is inefficient. Accordingly, there is a need in the art for improved standards.

SUMMARY OF THE INVENTION

[0006] Provided herein is a vector comprising amplicons of each of a plurality of nucleic acids, which may be selected from the group consisting of human or monkey T cell receptor excision circles (TREC), HIV-1 gag, SIVmac239/251, human or monkey albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human C-C chemokine receptor type 5 (CCR5). Each amplicon may be at least 18 nucleotides in length, or may be at least 50 nucleotides in length. The amplicon of human TREC may comprise the sequence set forth in SEQ ID NO: 31; the amplicon of HIV-1 gag may comprise the sequence set forth in SEQ ID NO: 32; the amplicon of SIVmac239/251 may comprise the sequence set forth in SEQ ID NO: 33; the amplicon of monkey albumin may comprise the sequence set forth in SEQ ID NO: 35; the amplicon of bacterial 16S ribosomal DNA may comprise the sequence set forth in SEQ ID NO: 36; the amplicon of SIVsm may comprise the sequence set forth in SEQ ID NO: 37; the amplicon of SIVagm may comprise the sequence set forth in SEQ ID NO: 38; and, the amplicon of human CCR5 may comprise the sequence set forth in SEQ ID NO: 34; or for each amplicon, a sequence substantially identical thereto. The sequence of each amplicon may consist of the foregoing respective sequence identifiers.

[0007] The amplicons may be arranged sequentially or contiguously. Each amplicon may be flanked by a restriction site that is not shared by any other amplicon in the vector. The vector may comprise amplicons of each of human TREC, HIV-1 gag, SIVmac239/251, monkey

albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human CCR5. The amplicons in the vector may consist of human TREC, HIV-1 gag, SIVmac239/251, monkey albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human CCR5. The vector may be a plasmid, which may further comprise one or more of an origin of replication and a selectable marker.

[0008] Also provided herein is a method of detecting HIV or SIV in a biological sample. The method may comprise (a) performing quantitative polymerase chain reactions (PCR) to amplify the amplicons of a vector described herein, wherein each PCR reaction comprises hybridizing each amplicon with a respective forward and reverse primer; (b) quantifying the amplification products of step (a); and, (c) comparing the amounts of amplified products quantified in step (b) to the amounts of amplification products produced by performing PCR on a biological sample from a subject using the forward and reverse primers of step (a); wherein the comparison of step (c) is indicative of the amount of HIV or SIV in the biological sample. In step (a), the PCR may be performed on serial dilutions of a known quantity of the vector. The serial dilutions may comprise 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}. The quantity of the vector may be about 20 μg.

[0009] The method may further comprise generating a standard curve based on the amounts of amplified products quantified in step (b) for each serial dilution of the vector by using least squares linear regression of the logarithm of vector copy number versus cycle threshold number. The comparing of step (c) may comprise using the standard curve best fit line. Each PCR reaction of step (a) may further comprise hybridizing the amplification products with a probe, and the quantifying of step (b) comprises detecting the amount of each probe. Each probe may comprise a 5'-fluorophore and a 3'-Black Hole Quencher.

[0010] The vector of the method may be a vector in which the sequences of the amplicons consist of SEQ ID NOs: 31-38. The forward and reverse primers may individually comprise the sequences set forth in SEQ ID NOs: 1 and 2; 4 and 5; 7 and 8; 10 and 11; 13 and 14; 16 and 17; 19 and 20; 22 and 23; 25 and 26; or, 28 and 29; or for each sequence, a sequence substantially identical thereto. The probes may comprise the sequence set forth in SEQ ID NO: 3, 6, 9, 12, 15, 18, 21, 24, 27, or 30; or for each sequence, a sequence substantially identical thereto.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0012] FIG. 1 shows a map of an exemplary plasmid indicating DNA sequences for quantification, flanking restriction enzymes, and functional plasmid components.

[0013] FIG. 2 shows amplification curves of an exemplary plasmid with TREC, HIVgag, SIVmac239, CCR5, albumin, 16S, SIVsm, SIVagm, and AmpR primers and probes. Amplification curves were normalized by feature scaling reporter values from 0 to 1 for each reaction.

DETAILED DESCRIPTION

[0014] Described herein is the creation and standardization of a vector that includes a plurality of amplicons commonly measured in microbiology studies: SIVmac239/251, SIVsm, SIVagm, HIV-1, bacterial 16S DNA, TREC, albumin, and CCR5 (albumin and/or CCR5 can be used to determine human and nonhuman primate cell number equivalents in each PCR reaction). Serial dilutions of this vector were made, and quantities were determined by comparison of dilutions of extracted DNA from known cell counts. Ordinarily, qPCR standard curve quantification involves use of one vector per amplicon, so that each vector construct must be individually sampled, purified, serially diluted and quantified in quantifying the amount of target sequences in an unknown biological sample. When quantifying multiple amplicons simultaneously with qPCR, it is often desirable to normalize individual qPCR standards to one another.

[0015] Unlike previous methods that require separate vector constructs for each target sequence, the compositions and methods provided herein incorporate multiple amplicons into a single vector construct. The inventors have discovered that this ensures equivalent template copy numbers for all amplicons. In addition, amplifying, purifying, diluting, and quantifying one vector construct instead of individual constructs streamlines standard curve qPCR analysis, thereby significantly reducing the amount of reagents required by $7/8^{th}$, and the space and time required for optimization; and, simplifying normalization between amplicons. The inventors have also discovered that the single vector construct works as well as well as individual constructs.

1. Definitions.

[0016] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting. As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise.

[0017] For recitation of numeric ranges herein, each intervening number there between with the same degree of precision is explicitly contemplated. For example, for the range of 6-9, the numbers 7 and 8 are contemplated in addition to 6 and 9, and for the range 6.0-7.0, the numbers 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6,9, and 7.0 are explicitly contemplated.

[0018] An "amplicon" as used herein may mean a portion of DNA or RNA that is the source or product of an amplification reaction such as PCR.

[0019] "Biological sample" as used herein may mean a sample of biological tissue or fluid that comprises nucleic acids. Such samples include, but are not limited to, tissue or fluid isolated from animals. Biological samples may also include sections of tissues such as biopsy and autopsy samples, frozen sections taken for histologic purposes, hair, and skin. Biological samples also include explants and primary and/or transformed cell cultures derived from animal or patient tissues. Biological samples may also be blood, a blood fraction, plasma, serum, urine, pleural effusion, mucus, ascitic fluid, amniotic fluid, stool, tears, saliva, cerebrospinal fluid, cervical secretions, vaginal secretions, endometrial secretions, gastrointestinal secretions, bronchial secretions, sputum, secretions from ovarian cyst, sperm, secretions from the breast, cell line, or tissue sample. A biological sample may be provided by removing a sample of cells from an animal, but can also be accomplished by using previously isolated cells (e.g., isolated by another person, at another time, and/or for another purpose), or by performing the methods described herein in vivo. Archival tissues, such as those having treatment or outcome history (e.g., formalin fixed, paraffin-embedded (FFPE) tissues), may also be used.

[0020] "Complement" or "complementary" as used herein to refer to a nucleic acid may mean Watson-Crick (e.g., A-T/U and C-G) or Hoogsteen base pairing between nucleotides or nucleotide analogs of nucleic acid molecules.

[0021] "Detection" may mean detecting the presence of a component in a sample. Detection may also mean detecting the absence of a component. Detection may also mean measuring the level of a component, either quantitatively or qualitatively.

[0022] "FRODO" as used herein, may stand for "FRugally Optimized DNA Octomer," and may be a vector as described herein, which contains amplicons for 8 different target genes. An example of FRODO is provided in FIG. 1.

[0023] "Gene" used herein may be a natural (e.g., genomic) or synthetic gene comprising transcriptional and/or translational regulatory sequences and/or a coding region and/or non-translated sequences (e.g., introns, 5'- and 3'-untranslated sequences). The coding region of a gene may be a nucleotide sequence coding for an amino acid sequence or a functional RNA, such as tRNA, rRNA, catalytic RNA, siRNA, miRNA or antisense RNA. A gene may also be an mRNA or cDNA corresponding to the coding regions (e.g., exons and miRNA) optionally comprising 5'- or 3'-untranslated sequences linked thereto. A gene may also be an amplified nucleic acid molecule produced in vitro comprising all or a part of the coding region and/or 5'- or 3'-untranslated sequences linked thereto.

[0024] "Identical" or "identity" as used herein in the context of two or more nucleic acids or polypeptide sequences may mean that the sequences have a specified percentage of residues that are the same over a specified region. The percentage may be calculated by optimally aligning the two sequences, comparing the two sequences over the specified region, determining the number of positions at which the identical residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the specified region, and multiplying the result by 100 to yield the percentage of sequence identity. In cases where the two sequences are of different lengths or the alignment produces one or more staggered ends and the specified region of comparison includes only a single sequence, the residues of single sequence are included in the denominator but not the numerator of the calculation. When comparing DNA and RNA, thymine (T) and uracil (U) may be considered equivalent. Identity may be performed manually or by using a computer sequence algorithm such as BLAST or BLAST 2.0.

[0025] "Nucleic acid" or "oligonucleotide" or "polynucleotide" used herein may mean at least two nucleotides covalently linked together. The depiction of a single strand also defines the sequence of the complementary strand. Thus, a nucleic acid also encompasses the complementary strand of a depicted single strand. Many variants of a nucleic acid may be used for the same purpose as a given nucleic acid. Thus, a nucleic acid also encompasses substantially identical nucleic acids and complements thereof. A single strand provides a probe that may

hybridize to a target sequence under stringent hybridization conditions. Thus, a nucleic acid also encompasses a probe that hybridizes under stringent hybridization conditions.

[0026] Nucleic acids may be single stranded or double stranded, or may contain portions of both double stranded and single stranded sequence. The nucleic acid may be DNA, both genomic and cDNA, RNA, or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine and isoguanine. Nucleic acids may be synthesized as a single stranded molecule or expressed in a cell (in vitro or in vivo) using a synthetic gene. Nucleic acids may be obtained by chemical synthesis methods or by recombinant methods.

[0027] A nucleic acid will generally contain phosphodiester bonds, although nucleic acid analogs may be included that may have at least one different linkage, e.g., phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphosphoroamidite linkages and peptide nucleic acid backbones and linkages. Other analog nucleic acids include those with positive backbones; non-ionic backbones, and non-ribose backbones, including those described in U.S. Pat. Nos. 5,235,033 and 5,034,506, which are incorporated by reference. Nucleic acids containing one or more non-naturally occurring or modified nucleotides are also included within one definition of nucleic acids. The modified nucleotide analog may be located for example at the 5'-end and/or the 3'-end of the nucleic acid molecule. Representative examples of nucleotide analogs may be selected from sugar- or backbone-modified ribonucleotides. It should be noted, however, that also nucleobase-modified ribonucleotides, i.e. ribonucleotides, containing a non-naturally occurring nucleobase instead of a naturally occurring nucleobase such as uridines or cytidines modified at the 5-position, e.g. 5-(2-amino)propyl uridine, 5-bromo uridine; adenosines and guanosines modified at the 8-position, e.g. 8-bromo guanosine; deaza nucleotides, e.g. 7-deaza-adenosine; O- and N-alkylated nucleotides, e.g. N6-methyl adenosine are suitable. The 2'-OH-group may be replaced by a group selected from H, OR, R, halo, SH, SR, NH2, NHR, NR2 or CN, wherein R is C1-C6 alkyl, alkenyl or alkynyl and halo is F, Cl, Br or I. Modified nucleotides also include nucleotides conjugated with cholesterol through, e.g., a hydroxyprolinol linkage as described in Krutzfeldt et al., Nature (Oct. 30, 2005), Soutschek et al., Nature 432:173-178 (2004), and U.S. Patent Publication No. 20050107325, which are incorporated herein by reference. Modified nucleotides and nucleic acids may also include locked nucleic

acids (LNA), as described in U.S. Patent No. 20020115080, which is incorporated herein by reference. Additional modified nucleotides and nucleic acids are described in U.S. Patent Publication No. 20050182005, which is incorporated herein by reference. Modifications of the ribose-phosphate backbone may be done for a variety of reasons, e.g., to increase the stability and half-life of such molecules in physiological environments, to enhance diffusion across cell membranes, or as probes on a biochip. Mixtures of naturally occurring nucleic acids and analogs may be made; alternatively, mixtures of different nucleic acid analogs, and mixtures of naturally occurring nucleic acids and analogs may be made.

[0028] "Substantially identical" used herein may mean that a first and second sequence are at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, 98% or 99% identical over a region of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20-40, 40-60, 60-100, or more nucleotides.

[0029] "Target nucleic acid" as used herein may mean a nucleic acid that may be bound by another nucleic acid. A target nucleic acid may be a DNA sequence. The target nucleic acid may be an RNA. One or more probes may bind the target nucleic acid.

[0030] "Vector" used herein may mean a nucleic acid sequence containing an origin of replication. A vector may be a plasmid, bacteriophage, bacterial artificial chromosome or yeast artificial chromosome. A vector may be a DNA or RNA vector. A vector may be either a self-replicating extrachromosomal vector or a vector which integrates into a host genome.

2. Vector

[0031] Provided herein is a vector comprising a plurality of portions (amplicons) of target nucleic acids, which may be suitable for use as a standard in qPCR (including RT-qPCR) for detecting or quantifying HIV or SIV in a biological sample. The target nucleic acids may include two or more of T cell receptor excision circles (TREC) (which may be human or monkey), HIV-1 gag, SIVmac239/251, albumin (which may be human or monkey), bacterial 16S ribosomal DNA, SIVsm (including SIVsmE543), SIVagm (including SIVagm155), and human C-C chemokine receptor type 5 (CCR5) (each, a "different target nucleic acid" or "different target"). In one example, the vector includes amplicons for all 8 of the foregoing different targets. The amplicons may be arranged sequentially in the vector, and in one example are arranged contiguously. Each amplicon may be flanked by restriction enzyme recognition sites that are unique to the amplicon, and which are absent elsewhere in the vector.

[0032] The amplicon may be contained in a sequence of a GenBank database accession described herein. The amplicon may be 18-250 nucleotide in length, and more specifically 18-200, 18-150, 18-100, 50-200, 50-150, or 100-200 nucleotides in length. Each amplicon may be capable of hybridizing to a respective forward and/or reverse primer described herein.

[0033] In one example, the TREC amplicon may be contained in a sequence of NCBI GenBank database Accession No. NG_001332.3. The HIVgag amplicon may be contained in a sequence of GenBank Accession No. NC_001802.1. The SIVmac239 amplicon may be contained in a sequence of GenBank Accession No. M33262.1. The albumin amplicon may be contained in a sequence of GenBank Accession No. MC_041758.1. The 16S amplicon may be contained in a sequence of GenBank Accession No. J01859.1. The SIVsm amplicon may be contained in a sequence of GenBank Accession No. U72748.2. The SIVagm amplicon may be contained in GenBank Accession No. M29975.1. The CCR5 amplicon may be contained in GenBank Accession No. AH005786.2.

[0034] Examples of amplicon sequences are provided in the following table.

Table 1

Amplicon	Sequence	SEQ ID NO:
hTREC	CACATCCCTTTCAACCATGCTGACACCTCTGGTTTTTGTAAAGGTGCCCACTCCTGTGCACGG TGATGCATAGGCACCTGCACCCCGTGCCTAAACCCTGCAGCTGGC	31
HIVgag	GGTGCGAGAGCGTCAGTATTAAGCGGGGGAGAATTAGATCGATGGGAAAAAATTCGGTTA AGGCCAGGGGGAAAGAAAAAATATAAATTAAAACATATAGTATGGGCAAGCAGGGAGCT	32
SIVmac239	GTCTGCGTCATCTGGTGCATTCACGCAGAAGAGAAAGTGAAACACACTGAGGAAGCAAAAC AGATAGTGCAGAGACACCTAGTG	33
hCCR5	CCAGAAGAGCTGAGACATCCGTTCCCCTACAAGAAACTCTCCCCGGTAAGTAACCTCTCAGCT GCTTGGCCTGTTAG	34
Monkey albumin	TGCATGAGAAAACGCCAGTAAGCGAGAAAGTCACCAAATGCTGCACGGAATCCTTGGTGAA CAGGCGACCAT	35
16S	CGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAA AGAAGTAGGTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGA AGTCGTAACAAGGTAACC	36
SIVsm (SIVsme543)	GGCAGGAAAATCCCTAGCAGGTTGGCGCCCGAACAGGGACTTGAAGGAGGTGAGAGCTCC TGAGTACGGCTGAGTGAAGGCAGTAAGGGC	37
SIVagm (SIVagmVer90)	GTCCAGTCTCAGCATTTACTTGCTGGGATATTGCAGCAGCAGAAGAATCTGCTGGCGGCTGT GGGAGCTCAACAGCAGATGTTGAAGCTGACCATTTGGGGTGTGAAAAACCTCAATGCCCG	38

[0035] The amplicon may also be substantially identical to one of the foregoing sequences. The amplicon may also comprise a truncation or extension of one of the foregoing by 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides, or a range of

the foregoing, at the 3' and/or 5' end. The amplicon may also comprise a sequence of 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, or 100 or more contiguous nucleotides contained in one of the foregoing sequences. In one example, the vector comprises the amplicons listed in Table 1, or sequences substantially identical thereto. In other example, the sequence of each of the amplicons respectively consists of the sequence provided in Table 1.

[0036] The vector may be a plasmid. Plasmids are well known in the art. The plasmid may comprise selectable marker. Selectable markers include a drug resistance gene, such as an antibiotic resistance gene, which may provide resistance to ampicillin or kanamycin. Other suitable selectable markers are known in the art. The plasmid may also comprise a multiple cloning site. Examples of suitable vectors include pUCIDT (Amp), pUCIDT (Kan), pIDTSmart (Amp), and, pBRIDT. In one example, the plasmid is pUCIDT (Amp). An example of the plasmid is provided in FIG. 1.

a. Primers

[0037] Provided herein are primers capable of hybridizing to a target nucleic acid or amplicon described herein, which may be contained in a biological sample or the vector. The primers may be used to amplify such nucleic acids. Each primer may have a sequence listed in Table 2, a sequence substantially identical thereto, or the complement thereof.

Table 2

	Primer/Probe Sequence	**SEQ ID NO:**
Human TREC		
Forward	5'- CACATCCCTTTCAACCATGCT -3'	1
Reverse	5'- GCCAGCTGCAGGGTTTAGG -3'	2
Probe	5'-ACACCTCTGGTTTTTGTAAAGGTGCCCACT-3'	3
Monkey TREC		
Forward	5'- CACATCCCTTTCAACCATGCT -3'	4
Reverse	5'- GCCAGCTGCAGGGTTTAGG -3'	5
Probe	5'-ACGCCTCTGGTTTTTGTAAAGGTGCTCACT-3'	6
HIV gag		
Forward	5'- GGTGCGAGAGCGTCAGTATTAAG -3'	7
Reverse	5'- AGCTCCCTGCTTGCCCATA -3'	8
Probe	5'-AAAATTCGGTTAAGGCCAGGGGGAAAGAA-3'	9
SIVmac239		
Forward	5'- GTCTGCGTCAT(T/C)TGGTGCATTC -3'	10
Reverse	5'- CACTAG(C/T)TGTCTCTGCACTAT(A/G)TGTTTTG -3'	11
Probe	5'-CTTC(A/G)TCAGT(C/T)TGTTTCACTTTCTCTTCTGCG-3'	12
Human Albumin		
Forward	5'- TGCATGAGAAAACGCCAGTAA -3'	13
Reverse	5'- ATGGTCGCCTGTTCACCAA -3'	14

Probe	5'-TGACAGAGTCACCAAATGCTGCACAGAA-3'	15
Monkey Albumin		
Forward	5'- TGCATGAGAAAACGCCAGTAA -3'	16
Reverse	5'- ATGGTCGCCTGTTCACCAA -3'	17
Probe	5'-AGAAAGTCACCAAATGCTGCACGGAATC-3'	18
16S (1369-1492)		
Forward	5'- CGGTGAATACGTTCYCGG -3'	19
Reverse	5'- GGWTACCTTGTTACGACTT -3'	20
Probe	5'-CTTGTACACACCGCCCGTC-3'	21
SIVsm		
Forward	5'- GGCAGGAAAATCCCTAGCAG -3'	22
Reverse	5'- GCCCTTACTGCCTTCACTCA -3'	23
Probe	5'-AGTCCCTGTTCRGGCGCCAA-3'	24
SIVagm		
Forward	5'- GTCCAGTCTCAGCATTTACTTG -3'	25
Reverse	5'- CGGGCATTGAGGTTTTTCAC -3'	26
Probe	5'-CAGATGTTGAAGCTGACCATTTGGG-3'	27
CCR5		
Forward	5'- CCAGAAGAGCTGAGACATCC -3'	28
Reverse	5'- CTCAGCTGCTTGGCCTRTTAG -3'	29
Probe	5'-TTCCCCTACAAGAAACTCTCCCCGGTAAGTA-3'	30

b. Probes

[0038] Also provided herein are probes capable of hybridizing to an amplification product generated by performing PCR on a target nucleic acid or amplicon described herein. Each probe may comprise a sequence listed in Table 1. The probe may also comprise a sequence substantially identical thereto, or the complement thereof.

[0039] A probe disclosed herein may comprise a label at the 5' or 3' end. In one example the label may be comprise a fluorophore. The fluorophore may be a fluorescein containing molecule, such as fluorescein amidite (FAM) (including 6-FAM), JOE, TET, Cal Fluor Gold 540, HEX, Cal Fluor Orange, or TAMRA. In particular, the label may be FAM, and in one example is 5'-FAM.

[0040] The probe may also comprise a quencher at the 5' or 3' end. The quencher may be fluorescent or non-fluorescent. The fluorescent quencher may be TAMRA. The non-fluorescent quencher may be a Black Hole Quencher (BHQ), such as BHQ-1, BHQ-2, or BHQ-3. In one example, the quencher is 3'-BHQ. More specifically, the probe may comprise 5'-FAM and 3'-BHQ.

3. Methods of detection

[0041] Provided herein are methods of detecting or quantifying the amount of a plurality of target nucleic acids or amplicons described herein. Also provided herein are methods of detecting HIV or SIV. The quantification method may comprise use of qPCR or RT-qPCR, which are well known in the art. In one example, the method comprises amplifying the target nucleic acids in a biological sample, and quantifying the amplification products. The method may also comprise performing the PCR using a vector described herein as a standard, and may further comprise generating a standard curve based on the amount of targets or amplicons amplified from the vector. Generating a standard curve may comprise performing PCR on serial dilutions of a known quantity of the vector, and quantifying the amount of targets or amplicons amplified from each dilution. The quantities of amplification products for each target from the biological sample may be compared to the quantities of amplification products for each target from the serial dilutions of the vector. Methods of calculating DNA copy numbers are known in the art. DNA copy numbers for target nucleic acids of the biological sample may be calculated using the standard curve best fit line. Relative copy numbers may also be calculated using the delta CT method.

[0042] Methods of generating standard curves in qPCR are well known in the art. In one example, least squares linear regression of the logarithm of vector copy number versus cycle threshold number is used to calculate a standard curve for serial dilutions of a known quantity of the vector. The dilutions may include one or more of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-8}, 10^{-9}, 10^{-10}, 10^{-11}, and 10^{-12}. In one example, the dilutions are 10^{-4} - 10^{-9}. The serial dilutions may be based on a known quantity of the vector, which may be from 0.1 μg-1 g, or may be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1000 μg, or a range of the foregoing. Suitable quantities are known in the art. The molecular weight of the plasmid coupled with measurement of absorbance at 280nm may also be used to determine concentration.

[0043] To yield absolute quantities of copies of targets described herein, serial dilutions of known quantities of the amplicons from the vector may be simultaneously analyzed with nucleic acids from the biological sample, and standard curves of cycle threshold versus analyte quantity may be created (as described in D. C. Douek et al., 1998, the contents of which are incorporated herein by reference). Known levels of amplicons may be created by transforming competent

bacteria with the vector, PCR amplification of the vector, and subsequent purification and quantification of the amplification products.

[0044] The method may comprise contacting the vector with one or more primers described herein, which may allow the one or more primers to hybridize with the vector. The method may also comprise detecting or quantifying PCR products using probes described herein, which may hybridize with an amplification product produced by a PCR method described herein. The method may comprise contacting amplification products produced by amplifying the vector or nucleic acids in the biological sample with one or more probes described herein.

[0045] The method of detection or quantification may also be performed on a biological sample. The biological sample may from a subject. The subject may be an animal. In particular, the subject may be a human or a non-human primate, including a monkey, orangutan, chimpanzee, or gorilla.

4. Kits

[0046] Provided herein is a kit containing a vector described herein. The kit may further include one or more primers and/or probes described herein. In one example, the kit includes the primers described in Table 1 above. In another example, the primers and probes described in Table 2 are used. The kit may include instructions for performing qPCR using the vector, primers, and probes for quantifying HIV and/or SIV in a biological sample. The instructions may include directions for generating a standard curve using the vector.

Example 1

Quantitative PCR measurement of HIV and SIV

[0047] This example demonstrates an exemplary vector described herein, and its use in quantifying HIV and SIV. In particular, here we describe creation and standardization of one plasmid construct which includes 8 amplicons commonly measured in microbiology studies: SIVmac239/251, SIVsm, SIVagm, HIV-1, bacterial 16S DNA, TREC, albumin, and CCR5 (albumin and/or CCR5 can be used to determine human and nonhuman primate cell number equivalents in each PCR reaction). Serial dilutions of this plasmid were made, and quantities were determined by comparison of dilutions of extracted DNA from known cell counts.

Generation, purification, quantification, and storage of the plasmid

[0048] Sequences of selected amplicon regions spanning forward and reverse primer and probe binding sites were obtained from the NCBI GenBank database (TREC: NG_001332.3, HIVgag: NC_001802.1, SIVmac239: M33262.1, albumin: MC_041758.1, 16S: J01859.1, SIVsm: U72748.2, SIVagm: M29975.1, CCR5: AH005786.2). Restriction enzymes flanking each amplicon were selected for unique recognition sequences absent in the carrier plasmid. The combined sequence of amplicons (914 nucleotides) was synthesized through the Custom Gene Synthesis service from Integrated DNA Technologies and delivered in a cloning vector containing an ampicillin resistance selectable marker (pUCIDT (Amp)). The plasmid product was prepared, purified, and sequenced using Alta Biotech's eXPRESS Mega Prep Service. A map of FRODO is shown in Figure 1.

[0049] An aliquot of the plasmid megaprep was further purified using a modified QIAprep Spin Miniprep Kit (Qiagen) protocol. Approximately 20 µg of FRODO plasmid was diluted in 500 µL Buffer PB and loaded onto DNA-binding column with standard wash and elution steps. Eluate was diluted again in 500 µL Buffer PB and the spin column procedure was repeated for a total of 5 times. Final eluate was quantified and assessed for purity by spectrometry (DeNovix DS-11).

[0050] Purified plasmid DNA was serially diluted 10^{-2} to 10^{-11} and yeast tRNAs (ThermoFisher AM7119) were added to a final concentration of 1 µg/mL to each dilution before aliquoting and freezing at -80°C. Amplification of FRODO with all primer/probe sets of interest was verified using the qPCR protocol described here (Figure 2). Albumin and TREC sequences included in FRODO amplify effectively with both human-targeted and macaque-targeted primers due to sequence homology in the amplicon sequence between species (only human-targeted primers shown in Figure 2). Additionally, plasmid copy numbers of the FRODO dilutions were determined by qPCR standard curve analysis with a previously quantified plasmid standard containing the SIVagm standard.

Table 3 PPlasmid dilution copy numbers

FRODO dilution	Plasmid Copy Number
10^{-4}	7.45×10^{7}
10^{-5}	3.63×10^{6}
10^{-6}	2.36×10^{5}
10^{-7}	1.52×10^{4}
10^{-8}	1.12×10^{3}
10^{-9}	7.27×10^{1}
10^{-10}	5.75×10^{0}

Quantification of microbiological analytes using real-time PCR and FRODO plasmid standard curve

[0051] Quantitative PCR reactions were carried out on StepOnePlusTM Real-Time PCR System (Applied Biosystems) with TaqMan™ Gene Expression Master Mix (Applied Biosystems) and final primer and probe concentrations of 500 nmol and 250 nmol, respectively. Primer and probe sequences for each amplicon are included in the table below. Each 25 μL reaction contains 5 μL template or standard DNA. PCR cycles were performed with standard temperature ramp as follows for all primer/probe sets: 95°C for 10 minutes, 45 cycles of 15 seconds at 95°C followed by 60 seconds at 60°C. Least squares linear regression of the logarithm of FRODO plasmid copy number versus cycle threshold number was used to calculate a standard curve for FRODO dilutions 10^{-4} - 10^{-9}. DNA copy numbers for target amplicons of unknown samples were calculated using the standard curve best fit line.

Table 4 Primer and probe sequences

Human TREC	
Forward	5'- CACATCCCTTTCAACCATGCT -3'
Reverse	5'- GCCAGCTGCAGGGTTTAGG -3'
Probe	5'- FAM - ACACCTCTGGTTTTTGTAAAGGTGCCCACT -BHQ -3'
Monkey TREC (Sodora et al., 2002)	
Forward	5'- CACATCCCTTTCAACCATGCT -3'
Reverse	5'- GCCAGCTGCAGGGTTTAGG -3'
Probe	5'- FAM - ACGCCTCTGGTTTTTGTAAAGGTGCTCACT -BHQ -3'
HIV gag (D.C. Douek et al., 2002)	
Forward	5'- GGTGCGAGAGCGTCAGTATTAAG -3'
Reverse	5'- AGCTCCCTGCTTGCCCATA -3'
Probe	5'- FAM - AAAATTCGGTTAAGGCCAGGGGGAAAGAA -BHQ -3'
SIVmac239	

(Mattapallil et al., 2005)	
Forward	5′- GTCTGCGTCAT(T/C)TGGTGCATTC -3′
Reverse	5′- CACTAG(C/T)TGTCTCTGCACTAT(A/G)TGTTTTG -3′
Probe	5′- FAM - CTTC(A/G)TCAGT(C/T)TGTTTCACTTTCTCTTCTGCG -BHQ -3′
Human Albumin (D.C. Douek et al., 2002)	
Forward	5′- TGCATGAGAAAACGCCAGTAA -3′
Reverse	5′- ATGGTCGCCTGTTCACCAA -3′
Probe	5′- FAM - TGACAGAGTCACCAAATGCTGCACAGAA -BHQ -3′
Monkey Albumin (Mattapallil et al., 2005)	
Forward	5′- TGCATGAGAAAACGCCAGTAA -3′
Reverse	5′- ATGGTCGCCTGTTCACCAA -3′
Probe	5′- FAM - AGAAAGTCACCAAATGCTGCACGGAATC -BHQ -3′
16S (1369-1492) (Suzuki, Taylor, & DeLong, 2000)	
Forward	5′- CGGTGAATACGTTCYCGG -3′
Reverse	5′- GGWTACCTTGTTACGACTT -3′
Probe	5′- FAM - CTTGTACACACCGCCCGTC -BHQ -3′
SIVsm (Brenchley et al., 2012)	
Forward	5′- GGCAGGAAAATCCCTAGCAG -3′
Reverse	5′- GCCCTTACTGCCTTCACTCA -3′
Probe	5′- FAM - AGTCCCTGTTCRGGCGCCAA -BHQ -3′
SIVagm (Beaumier et al., 2009)	
Forward	5′- GTCCAGTCTCAGCATTTACTTG -3′
Reverse	5′- CGGGCATTGAGGTTTTTCAC -3′
Probe	5′- FAM - CAGATGTTGAAGCTGACCATTTGGG -BHQ -3′
CCR5 (Venneti et al., 2008)	
Forward	5′- CCAGAAGAGCTGAGACATCC -3′
Reverse	5′- CTCAGCTGCTTGGCCTRTTAG -3′
Probe	5′- FAM - TTCCCCTACAAGAAACTCTCCCCGGTAAGTA -BHQ -3′

The nucleic acid sequences are SEQ ID NOs: 1-30, respectively.

Literature Cited (the contents of which are incorporated by reference)

[0052] Beaumier, C. M., Harris, L. D., Goldstein, S., Klatt, N. R., Whitted, S., McGinty, J., . . . Brenchley, J. M. (2009). CD4 downregulation by memory CD4+ T cells in vivo renders African green monkeys resistant to progressive SIVagm infection. Nat Med, 15(8), 879-885. Retrieved

from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19525963. doi:nm.1970 [pii] 10.1038/nm.1970

[0053] Brenchley, J. M., Hill, B. J., Ambrozak, D. R., Price, D. A., Guenaga, F. J., Casazza, J. P., . . . Koup, R. A. (2004). T-Cell Subsets That Harbor Human Immunodeficiency Virus (HIV) In Vivo: Implications for HIV Pathogenesis. J Virol, 78(3), 1160-1168. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=14722271.

[0054] Brenchley, J. M., Vinton, C., Tabb, B., Hao, X. P., Connick, E., Paiardini, M., . . . Estes, J. D. (2012). Differential infection patterns of CD4+ T cells and lymphoid tissue viral burden distinguish progressive and nonprogressive lentiviral infections. Blood, 120(20), 4172- 4181. Retrieved from www.ncbi.nlm.nih.gov/pubmed/22990012. doi:10.1182/blood-2012-06-437608.

[0055] Chomont, N., El-Far, M., Ancuta, P., Trautmann, L., Procopio, F. A., Yassine-Diab, B., . . . Sekaly, R. P. (2009). HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. Nat Med, 15(8), 893-900. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19543283. doi:nm.1972 [pii] 10.1038/nm.1972.

[0056] Douek, D. (2004). Thymic output and HIV infection: on the right TREC. Immunity, 21(6), 744- 745. Retrieved from www.ncbi.nlm.nih.gov/pubmed/15589163. doi:10.1016/j.immuni.2004.11.005.

[0057] Douek, D. C., Betts, M. R., Hill, B. J., Little, S. J., Lempicki, R., Metcalf, J. A., . . . Koup, R. A. (2001). Evidence for increased T cell turnover and decreased thymic output in HIV infection. J Immunol, 167(11), 6663-6668. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11714838.

[0058] Douek, D. C., Brenchley, J. M., Betts, M. R., Ambrozak, D. R., Hill, B. J., Okamoto, Y., . . . Koup, R. A. (2002). HIV Preferentially Infects HIV-Specific CD4+ T-cells. Nature, 417(6884), 95-98. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=11986671.

[0059] Douek, D. C., McFarland, R. D., Keiser, P. H., Gage, E. A., Massey, J. M., Haynes, B. F., . . . Koup, R. A. (1998). Changes in thymic function with age and during the treatment of HIV infection. Nature, 396(6712), 690-695.

[0060] Douek, D. C., Vescio, R. A., Betts, M. R., Brenchley, J. M., Hill, B. J., Zhang, L., . . . Koup, R. A. (2000). Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. Lancet, 355(9218), 1875-1881. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=10866444.

[0061] Jiang, W., Lederman, M. M., Hunt, P., Sieg, S. F., Haley, K., Rodriguez, B., . . . Brenchley, J. M. (2009). Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-Treated HIV infection. J Infect Dis, 199(8), 1177-1185. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19265479. doi:10.1086/597476.

[0062] Klase, Z., Ortiz, A., Deleage, C., Mudd, J. C., Quinones, M., Schwartzman, E., . . . Brenchley, J. M. (2015). Dysbiotic bacteria translocate in progressive SIV infection. Mucosal Immunol, 8(5), 1009-1020. Retrieved from www.ncbi.nlm.nih.gov/pubmed/25586559. doi:10.1038/mi.2014.128.

[0063] Lai, S. H., Starke, C. E., Flynn, J. K., Vinton, C. L., Ortiz, A. M., Mudd, J. C., & Brenchley, J. M. (2019). Simian Immunodeficiency Virus Infects Functionally Polarized Memory CD4 T Cells Equivalently In Vivo. J Virol, 93(9). Retrieved from www.ncbi.nlm.nih.gov/pubmed/30787150. doi:10.1128/JVI.02163-18.

[0064] Mattapallil, J. J., Douek, D. C., Hill, B., Nishimura, Y., Martin, M., & Roederer, M. (2005). Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature, 434(7037), 1093-1097. Retrieved from www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=15793563.

[0065] McFarland, R. D., Douek, D. C., Koup, R. A., & Picker, L. J. (2000). Identification of a human recent thymic emigrant phenotype. Proc Natl Acad Sci U S A, 97(8), 4215-4220. Retrieved from www.ncbi.nlm.nih.gov/pubmed/10737767. doi:10.1073/pnas.070061597.

[0066] Muraro, P. A., Douek, D. C., Packer, A., Chung, K., Guenaga, F. J., Cassiani-Ingoni, R., . . . Martin, R. (2005). Thymic output generates a new and diverse TCR repertoire after autologous stem cell transplantation in multiple sclerosis patients. J Exp Med, 201(5), 805-816. Retrieved from www.ncbi.nlm.nih.gov/pubmed/15738052. doi:10.1084/jem.20041679.

[0067] Sodora, D. L., Milush, J. M., Ware, F., Wozniakowski, A., Montgomery, L., McClure, H. M., . . . Koup, R. A. (2002). Decreased levels of recent thymic emigrants in peripheral blood of simian immunodeficiency virus-infected macaques correlate with alterations within the thymus. J Virol, 76(19), 9981-9990. Retrieved from www.ncbi.nlm.nih.gov/pubmed/12208974. doi:10.1128/jvi.76.19.9981- 9990.2002.

[0068] Suzuki, M. T., Taylor, L. T., & DeLong, E. F. (2000). Quantitative analysis of small-subunit rRNA genes in mixed microbial populations via 5'-nuclease assays. Appl Environ Microbiol, 66(11), 4605-4614. Retrieved from www.ncbi.nlm.nih.gov/pubmed/11055900. doi:10.1128/aem.66.11.4605-4614.2000.

[0069] Tanaskovic, S., Fernandez, S., Price, P., Lee, S., & French, M. A. (2010). CD31 (PECAM-1) is a marker of recent thymic emigrants among CD4+ T-cells, but not CD8+ T-cells or gammadelta T-cells, in HIV patients responding to ART. Immunol Cell Biol, 88(3), 321- 327. Retrieved from www.ncbi.nlm.nih.gov/pubmed/20065992. doi:10.1038/icb.2009.108.

[0070] Venneti, S., Bonneh-Barkay, D., Lopresti, B. J., Bissel, S. J., Wang, G., Mathis, C. A., . . . Wiley, C. A. (2008). Longitudinal in vivo positron emission tomography imaging of infected and activated brain macrophages in a macaque model of human immunodeficiency virus encephalitis correlates with central and peripheral markers of encephalitis and areas of synaptic degeneration. Am J Pathol, 172(6), 1603-1616. Retrieved from www.ncbi.nlm.nih.gov/pubmed/18467697. doi:10.2353/ajpath.2008.070967.

CLAIMS

What is claimed is:

1. A vector comprising amplicons of each of a plurality of nucleic acids selected from the group consisting of human or monkey T cell receptor excision circles (TREC), HIV-1 gag, SIVmac239/251, human or monkey albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human C-C chemokine receptor type 5 (CCR5), wherein each portion is at least 18 nucleotides in length.

2. The vector of claim 1, wherein each amplicon is at least 50 nucleotides in length.

3. The vector of claim 1 or 2, wherein the amplicon of human TREC comprises the sequence set forth in SEQ ID NO: 31; the amplicon of HIV-1 gag comprises the sequence set forth in SEQ ID NO: 32; the amplicon of SIVmac239/251 comprises the sequence set forth in SEQ ID NO: 33; the amplicon of monkey albumin comprises the sequence set forth in SEQ ID NO: 35; the amplicon of bacterial 16S ribosomal DNA comprises the sequence set forth in SEQ ID NO: 36; the amplicon of SIVsm comprises the sequence set forth in SEQ ID NO: 37; the amplicon of SIVagm comprises the sequence set forth in SEQ ID NO: 38; and, the amplicon of human CCR5 comprises the sequence set forth in SEQ ID NO: 34; or for each amplicon, a sequence substantially identical thereto.

4. The vector of claim 3, wherein the sequence of the amplicon of human TREC consists of the sequence set forth in SEQ ID NO: 31; the sequence of the amplicon of HIV-1 gag consists of the sequence set forth in SEQ ID NO: 32; the sequence of the amplicon of SIVmac239/251 consists of the sequence set forth in SEQ ID NO: 33; the sequence of the amplicon of monkey albumin consists of the sequence set forth in SEQ ID NO: 35; the sequence

of the amplicon of bacterial 16S ribosomal DNA consists of the sequence set forth in SEQ ID NO: 36; the sequence of the amplicon of SIVsm consists of the sequence set forth in SEQ ID NO: 37; the sequence of the amplicon of SIVagm consists of the sequence set forth in SEQ ID NO: 38; and, the sequence of the amplicon of human CCR5 consists of the sequence set forth in SEQ ID NO: 34.

5. The vector of any one of claims 1-4, wherein the amplicons are arranged sequentially or contiguously.

6. The vector of any one of claims 1-5, comprising amplicons of each of human TREC, HIV-1 gag, SIVmac239/251, monkey albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human CCR5.

7. The vector of claim 6, wherein the amplicons in the vector consist of human TREC, HIV-1 gag, SIVmac239/251, monkey albumin, bacterial 16S ribosomal DNA, SIVsm, SIVagm, and human CCR5.

8. The vector of any one of claims 1-7, wherein the vector is a plasmid further comprising one or more of an origin of replication and a selectable marker.

9. A method of detecting HIV or SIV in a biological sample, comprising:

(a) performing quantitative polymerase chain reactions (PCR) to amplify the amplicons of the vector of any one of claims 1-8, wherein each PCR reaction comprises hybridizing each amplicon with a respective forward and reverse primer;

(b) quantifying the amplification products of step (a); and,

(c) comparing the amounts of amplified products quantified in step (b) to the amounts of amplification products produced by performing PCR on a biological sample from a subject using the forward and reverse primers of step (a);

wherein the comparison of step (c) is indicative of the amount of HIV or SIV in the biological sample.

10. The method of claim 9, wherein in step (a), PCR is performed on serial dilutions of a known quantity of the vector.

11. The method of claim 10, wherein the serial dilutions comprise 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, and 10^{-9}.

12. The method of claim 11, wherein the quantity of the vector is about 20 μg.

13. The method of claim 10, further comprising generating a standard curve based on the amounts of amplified products quantified in step (b) for each serial dilution of the vector by using least squares linear regression of the logarithm of vector copy number versus cycle threshold number.

14. The method of claim 13, wherein the comparing of step (c) comprises using the standard curve best fit line.

15. The method of any one of claims 9-14, wherein each PCR reaction of step (a) further comprises hybridizing the amplification products with a probe, and the quantifying of step (b) comprises detecting the amount of each probe.

16. The method of claim 13, wherein each probe comprises a 5'-fluorophore and a 3'-Black Hole Quencher.

17. The method of any one of claims 9-16, wherein the vector is the vector of claim 6, and the forward and reverse primers individually comprise the sequences set forth in SEQ ID NOs: 1 and 2; 4 and 5; 7 and 8; 10 and 11; 13 and 14; 16 and 17; 19 and 20; 22 and 23; 25 and 26; or, 28 and 29; or for each sequence, a sequence substantially identical thereto.

18. The method of any one of claims 9-17, wherein the probes comprise the sequence set forth in SEQ ID NO: 3, 6, 9, 12, 15, 18, 21, 24, 27, or 30; or for each sequence, a sequence substantially identical thereto.

FIG. 1

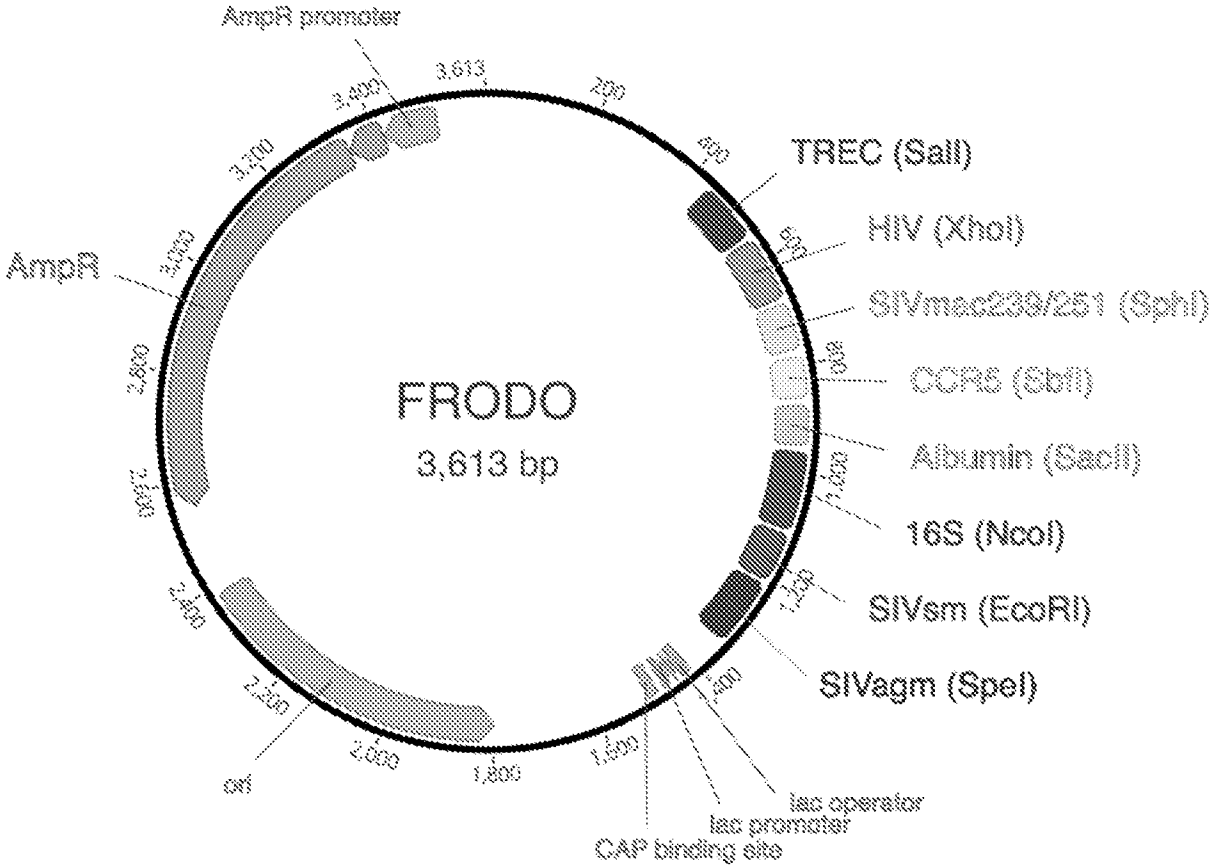
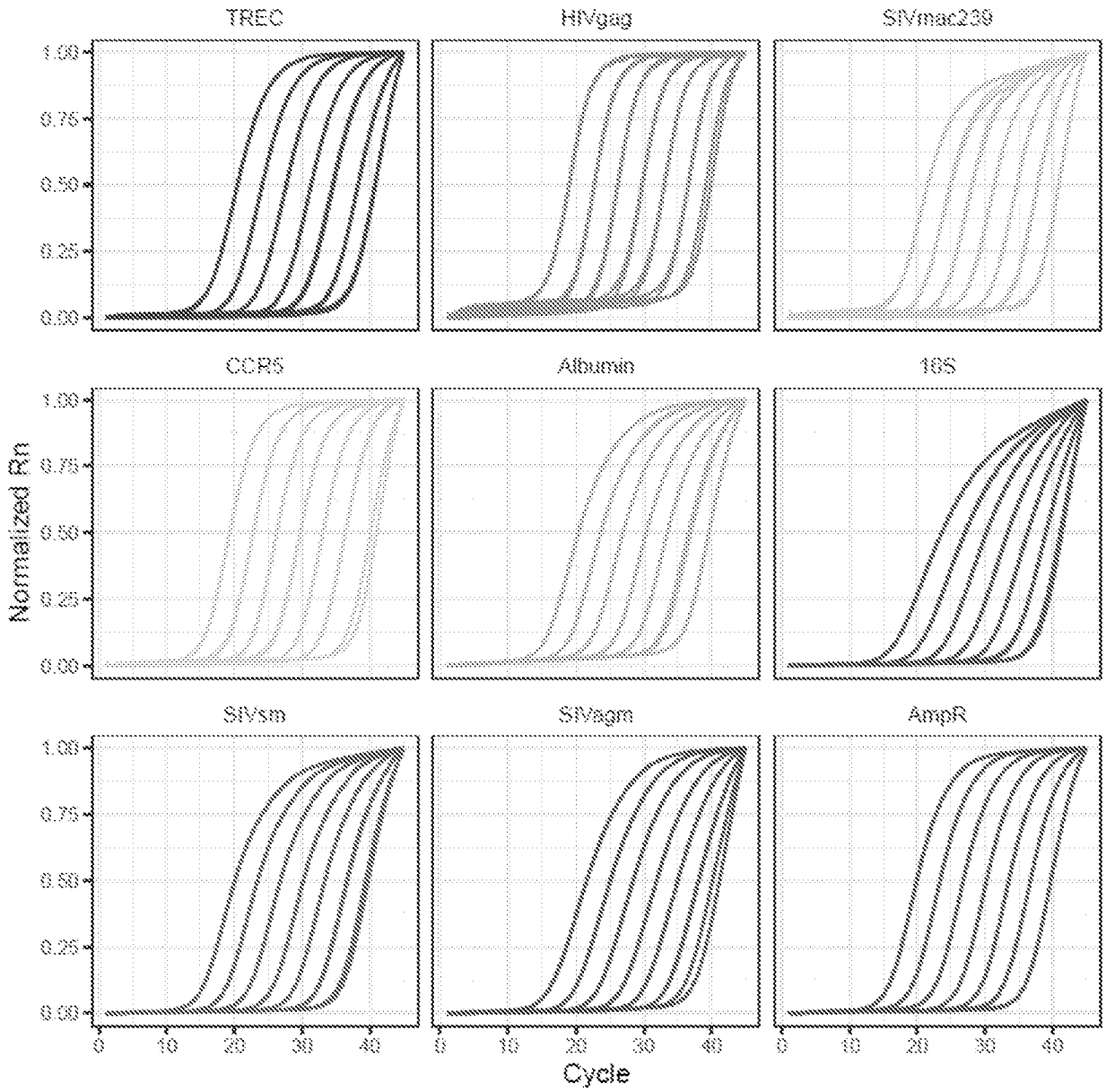

AmpR promoter
3,613
200
400
TREC (SalI)
600
HIV (XhoI)
800
SIVmac239/251 (SphI)
1,000
CCR5 (EbfI)
1,200
Albumin (SacII)
1,400
16S (NcoI)
1,600
SIVsm (EcoRI)
1,800
SIVagm (SpeI)
2,000
2,200
2,400
2,600
2,800
3,000
3,200
3,400
AmpR
ori
FRODO
3,613 bp
lac operator
lac promoter
CAP binding site

FIG. 2


TREC
HIVgag
SIVmac239
CCR5
Albumin
16S
SIVsm
SIVagm
AmpR
Normalized Rn
1.00
0.75
0.50
0.25
0.00
0
10
20
30
40
Cycle

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2021/063514

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12Q1/6851 C12Q1/686 C12Q1/70
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2009/004643 A1 (ECKER DAVID J [US] ET AL) 1 January 2009 (2009-01-01) paragraph [0018]	1-18
A	CN 111 139 317 A (EUROFINS TECH SERVICE COMPANY LIMITED) 12 May 2020 (2020-05-12) Summary	1-18
A	CN 109 182 600 A (HEFEI INST PHYSICAL SCI CAS; HEFEI ZHONGKE YIKANGDA BIOMEDICAL CO LTD) 11 January 2019 (2019-01-11) Summary	1-18

[ ] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 April 2022	03/05/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Sprinks, Matthew

1

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2021/063514

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed:

      [X] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [ ] furnished subsequent to the international filing date for the purposes of international search only:

      [ ] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2021/063514

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2009004643 A1	01-01-2009	US 2009004643 A1	01-01-2009
		US 2012107795 A1	03-05-2012
		US 2014141502 A1	22-05-2014
		WO 2005094421 A2	13-10-2005
CN 111139317 A	12-05-2020	NONE	
CN 109182600 A	11-01-2019	NONE	