(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 September 2009 (24.09.2009)

PCT

(10) International Publication Number
WO 2009/117537 A1

(51) International Patent Classification:
C12Q 1/68 (2006.01)

(21) International Application Number:
PCT/US2009/037593

(22) International Filing Date:
19 March 2009 (19.03.2009)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/038,064 20 March 2008 (20.03.2008) US
61/054,191 19 May 2008 (19.05.2008) US

(71) Applicant *(for all designated States except US)*: **SIEMENS HEALTHCARE DIAGNOSTICS INC.** [US/US]; 511 Benedict Ave., New York, NY 10591 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **DETMER, Jill** [US/US]; 18 Avon Road, Kensington, CA 94707 (US). **JIANG, Xiaoqiao** [US/US]; 4524 Shellflower Court, Concord, CA 94518 (US). **LE, Minh** [US/US]; 1519 Liberty St., El Cerrito, CA 94530 (US). **SHERMAN, David** [US/US]; 2948 Ponteverde Lane, Davis, CA 95618 (US).

(74) Agent: **SIEMENS CORPORATION**; Intellectual Property Dept., 170 Wood Avenue South, Iselin, NY 08830 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

— *with sequence listing part of description (Rule 5.2(a))*

(54) **Title**: NON-COMPETITIVE INTERNAL CONTROLS FOR USE IN NUCLEIC ACID TESTS

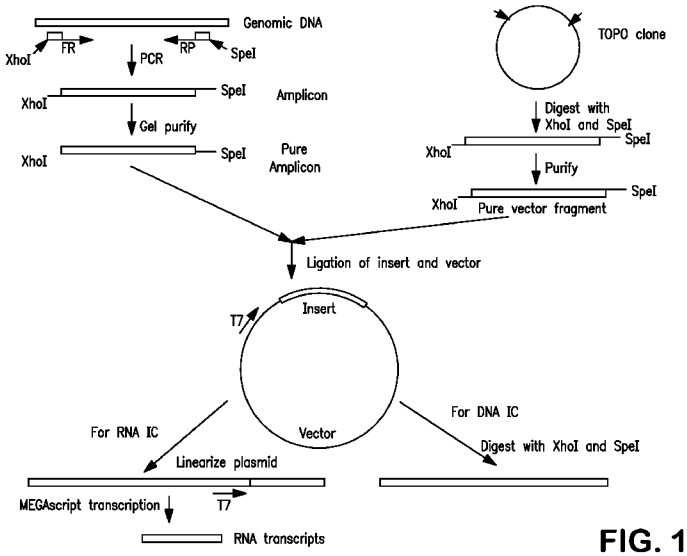

FIG. 1

(57) **Abstract**: Provided are non-competitive internal controls for use in nucleic acid tests (NATs), which are obtained from the organisms *Methanobacterium thermoautrophicum* (MET) and *Zea mays* (Corn). The non-competitive internal controls have utility in DNA and RNA NATs selected from Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhea* (GC), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency Virus I (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

WO 2009/117537 A1

NON-COMPETITIVE INTERNAL CONTROLS FOR USE IN NUCLEIC ACID TESTS

TECHNICAL FIELD

[0001] This application relates generally to tools for conducting diagnostic assays and more specifically to internal control sequences for use in nucleic acid tests (NATs) that do not compete with the target nucleic acid sequences.

BACKGROUND OF THE INVENTION

[0002] In order to ensure that nucleic acid tests (NATs) are properly performed, the assays require the presence of internal controls. In diagnostic NATs, the presence of an internal control can guarantee the integrity of the test. Specifically, by including an internal control in a NAT, samples testing positive for the internal control and the target nucleic acid are true positives. By contrast, samples testing only for the internal control are true negatives, samples testing only for the target nucleic acid are true negatives, and samples having no detectable internal control or target are false negatives.

[0003] The most commonly used diagnostic assay requiring the presence of internal controls is the polymerase chain reaction (PCR) assay, which is a target amplification assay in its traditional use and a target amplification and quantification assay in its modified use. There are several types of PCR assays: traditional PCR (amplification of DNA); reverse-transcriptase PCR (also known as "RT-PCR"; amplification of DNA using RNA as a starting material), real-time PCR (simultaneous quantification and amplification of DNA); and real-time RT-PCR (simultaneous quantification and amplification of DNA using RNA as a starting material).

[0004] In amplification assays, such as PCR assays, typically, one of two types of internal controls is used: competitive internal controls and non-competitive internal controls.

[0005] With competitive internal controls, the target and the internal control are amplified with one common set of primers under the same conditions and in the same PCR tube. With competitive internal controls, the internal control nucleic acid is flanked by the same primer sequence that is used to initiate amplification of the target nucleic acid. When the PCR assay is performed correctly, the IC nucleic acid will be detected during post amplification analysis. As is clear from its name, competitive internal controls are based on competition between target DNA and the internal control. For competitive internal controls to be effective, the

amount of internal control in the sample tube is critical to the detection limit. A disadvantage of the use of competitive internal controls is based upon its structure, specifically, simultaneous amplification of two different nucleic acid fragments flanked by the same primer sites risks inhibition or enhancement of one or both products depending on the molar ratio, the length, the sequence, and the secondary structure of the nucleic acid fragments. Another disadvantage of competitive internal controls is that they are incapable for use in multiplex assays, which screen multiple targets in a single assay.

[0007] With non-competitive internal controls, the target and the internal control are amplified using a different primer set for each; thus, non-competitive internal controls require a PCR in which two reactions with different kinetics proceed simultaneously. Because the kinetics of the two reactions is different, there is no competition for the primers. Non-competitive internal control primer sets currently in use typically target genes other than the target gene (e.g., encoding rRNA), which are present in a sample in higher copy number than the target gene. The most commonly used non-competitive internal control in the art uses primers specific to conserved sequences of 16S and 23S ribosomal DNA. There remains a need in the art for additional non-competitive internal controls that may be prepared for use in multiple assays. An advantage of non-competitive internal controls is that unlike competitive internal controls, non-competitive internal controls they may be stored for use in multiple reactions and also may be used in multiplex reactions. There is a need in the art for such a non-competitive internal control.

SUMMARY OF THE INVENTION

[0008] The present invention overcomes the need in the art for a non-competitive internal control for use in NATs by providing nucleic acid sequences that may be prepared in the lab and stored for use in multiple reactions and in multiplex NATs.

[0009] In one aspect of the invention, there is provided a non-competitive internal control for use in nucleic acid tests (NATs) comprising a nucleic acid obtained from an organism selected from *Methanobacterium thermoautrophicum* (MET) and *Zea mays*.

[0010] In one embodiment of the invention, the non-competitive internal controls are comprised of DNA and are used in DNA NATs selected from the group consisting of Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (GC [for *gonococci*]), and Hepatitis B virus (HBV).

[0011] In another embodiment of the invention, the non-competitive internal control are comprised of RNA and are used in RNA NATs selected from the group consisting Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

[0012] In another aspect of the invention, there is provided, a method of preparing a non-competitive internal control for use in nucleic acid tests (NATs), comprising the steps of: (a) extracting genomic DNA from *Methanobacterium thermoautrophicum* (MET); (b) generating an amplicon from the genomic DNA of step (a) using forward and reverse primers having at least two restriction enzyme sites and at least one target specific sequence; (c) generating a plasmid by ligating the amplicon of step (b) with a vector sequence having a promoter sequence and restriction enzyme sites that are identical to the restriction enzyme sites of the amplicon; and (d) digesting the plasmid with restriction enzymes corresponding to the restriction enzyme sites of steps (b) and (c) to generate MET internal control DNA.

[0013] Where RNA is required, the method further comprises the step of (e): preparing MET internal control RNA from the DNA of step (d).

[0014] In a further aspect of the invention, there is provided a method of preparing a non-competitive internal control for use in nucleic acid tests (NATs), comprising the steps of: (a) extracting genomic DNA from *Zea Mays* (Corn); (b) generating an amplicon from the genomic DNA of step (a) using forward and reverse primers having at least two restriction enzyme sites and target specific sequence; (c) generating a plasmid by ligating the amplicon of step (b) with a vector sequence having a promoter sequence and restriction enzyme sites that are identical to the restriction enzyme sites of the amplicon; (d) digesting the plasmid with restriction enzymes corresponding to the restriction enzyme sites of steps (b) and (c) to generate Corn internal control DNA.

[0015] Where RNA is required, the method further comprises the step of: (e) preparing Corn internal control RNA from the DNA of step (d).

[0016] In one embodiment of the invention, the at least two restriction enzyme sites of steps (b) and (d) (for both MET and Corn) correspond to the sequences of restriction enzymes XhoI and SpeI.

[0017] In another embodiment of the invention, the promoter sequence of step (c) (for both MET and Corn) is a T7 promoter sequence.

[0018] In a further embodiment of the invention, the MET or Corn internal control DNA is used as an non-competitive internal control in DNA NATs selected from the group consisting of: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory

syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (GC), and Hepatitis B virus (HBV).

[0019] In yet another embodiment of the invention MET or Corn internal control RNA is used as a non-competitive internal control in RNA NATs selected from the group consisting of: Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

[0020] Additional aspects, advantages and features of the invention will be set forth, in part, in the description that follows, and, in part, will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **FIG. 1** is a schematic diagram of the method for preparing the internal control sequences of the present invention.

[0022] **FIG. 2** is a graph of the amplification plot for *Chlamydia trachomatis* (CT) (left curve) and MET IC (right curve) in a single well.

[0023] **FIG. 3** is a graph of the amplification plot for *Neisseria gonorrhea* (GC) (left curve) and MET IC (right curve) in a single well.

[0024] **FIG. 4** is a graph of the amplification plot for Hepatitis C virus (HCV) (left curve) and MET IC (right curve) in a single well.

DETAILED DESCRIPTION OF THE INVENTION

[0025] Set forth below is a description of what are currently believed to be the preferred embodiments and best examples of the claimed invention. Any alternates or modifications in function, purpose, or structure are intended to be covered by the claims of this application.

[0026] DEFINITIONS:

[0027] In describing and claiming the present invention, the following terminology the following definitions are used for the purpose of describing particular embodiments only, and is not intended to be limiting. As used in this specification and the appended claims, the singular forms “a,” “an,” and “the” include plural referents unless the context clearly dictates otherwise.

[0028] The term “non-competitive internal control” refers to an internal control nucleic acid sequence that includes primer sites that are not present in the target nucleic acid sequence.

[0029] The term "competitive internal control" refers to an internal control nucleic acid sequence that includes primer sites that are also present in the target nucleic acid sequence.

[0030] The terms "FW" and "FP" indicate forward primers and the terms "RV" and "RP" indicate reverse primers. The term "P" when used alone refers to a probe.

[0031] The term "PCR primer for the construction of internal control (IC) clone" refers to oligonucleotides that were designed to introduce unique sequences and restriction sites into a newly constructed IC plasmid DNA through overlapping PCR reactions.

[0032] The term "amplification primer" (also referred to herein as "primer") refers to an oligonucleotide that is complementary to DNA or RNA molecules and provides the 3 -OH-end of a substrate to which any DNA polymerase can add the nucleotides of a growing DNA chain in the 5 to 3 direction.

[0033] The term "detection probe" (also referred to herein as "probe") refers to an oligonucleotide capable of selectively hybridizing to the amplified target nucleic acid under appropriate conditions. The detection probe may consist of a nucleotide with 5 -reporter dye (R) and a 3 -quencher dye (Q). A fluorescent reporter dye and fluorophore or a quencher that is either red-shifted fluorescent or non-fluorescent may be covalently linked to the 5 -end or 3 -end of the oligonucleotide. The detection probe acts as a TAQMAN® (Applied Biosystems, Foster City, CA) probe or other detection probes, such as beacons, non-nuclease real time amplification probes during amplification and detection process.

[0034] The term "diagnostic target (unknown)" refers to the nucleic acid sequence(s) that the PCR assay has been designed to detect specifically. Examples of these assays include targets, such as for example, Hepatitis B Virus (HBV), Hepatitis C Virus (HCV), and Human Immunodeficiency Virus (HIV).

[0035] The term "diagnostic target (known)" refers to the unique DNA or RNA target that is spiked at a known concentration into either the extraction step or amplification mixture used to isolate and amplify the Specific Diagnostic Target whose presence or quantity in the sample is unknown. In addition, unique primers and probes that recognize the unique fragments of RNA or DNA are added into the amplification mixture. These internal controls can be used to monitor the efficiency of the target extraction, amplification, and detection in real time kPCR assays.

[0036] As used herein, the term "target amplification" refers to enzyme-mediated procedures that are capable of producing billions of copies of nucleic acid target. Examples of enzyme-mediated target amplification procedures known in the art include PCR, nucleic acid-sequence-based amplification ("NASBA"), transcription-mediated amplification

("TMA"), strand displacement amplification ("SDA"), and ligase chain reaction ("LCR"). The most widely used target amplification procedure is PCR, first described for the amplification of DNA by Mullins et al. in U.S. Patent No. 4,683,195 and Mullis in U.S. Patent No. 4,683,202. The PCR procedure is well known to those of ordinary skill in the art. Where the starting material for the PCR reaction is RNA, complementary DNA ("cDNA") is made from RNA via reverse transcription. A PCR used to amplify RNA products is referred to as reverse transcriptase PCR or "RT-PCR."

[0037] In the PCR technique, a sample of DNA is mixed in a solution with a molar excess of two oligonucleotide primers of 10-30 base pairs each that are prepared to be complementary to the 3′ end of each strand of the DNA duplex; a molar excess of unattached nucleotide bases (i.e., dNTPs); and DNA polymerase, (preferably Taq polymerase, which is stable to heat), which catalyzes the formation of DNA from the oligonucleotide primers and dNTPs. Of the two primers, one is a forward primer that will bind in the 5′-3′ direction to the 3′ end of one strand of the denatured DNA analyte and the other is a reverse primer that will bind in the 3′-5′ direction to the 5′ end of the other strand of the denatured DNA analyte. The solution is heated to 94-96°C to denature the double-stranded DNA to single-stranded DNA. When the solution cools, the primers bind to the separated strands and the DNA polymerase catalyzes a new strand of analyte by joining the dNTPs to the primers. When the process is repeated and the extension products synthesized from the primers are separated from their complements, each extension product serves as a template for a complementary extension product synthesized from the other primer. In other words, an extension product synthesized from the forward primer, upon separation, would serve as a template for a complementary extension product synthesized from the reverse primer. Similarly, the extension product synthesized from the reverse primer, upon separation, would serve as a template for a complementary extension product synthesized from the forward primer. In this way, the region of DNA between the primers is selectively replicated with each repetition of the process. Since the sequence being amplified doubles after each cycle, a theoretical amplification of one billion copies may be attained after repeating the process for a few hours; accordingly, extremely small quantities of DNA may be amplified using PCR in a relatively short period of time.

[0038] As used herein, the term "amplicon" refers to amplified nucleic acid product, such as for example, amplified PCR product.

[0039] Where the starting material for the PCR reaction is RNA, complementary DNA ("cDNA") is made from RNA via reverse transcription. The resultant cDNA is then

amplified using the PCR protocol described above. Reverse transcriptases are known to those of ordinary skill in the art as enzymes found in retroviruses that can synthesize complementary single strands of DNA from an mRNA sequence as a template. The enzymes are used in genetic engineering to produce specific cDNA molecules from purified preparations of mRNA. A PCR used to amplify RNA products is referred to as reverse transcriptase PCR or "RT-PCR."

[0040] The terms "real-time PCR" and "real-time RT-PCR," also known in the art as "kinetic PCR" ("kPCR") or "kinetic RT-PCR" ("kRT-PCR"), refers to modified PCR assays that are used for simultaneous amplification and quantification of DNA. With real-time PCR, PCR products are detected via a fluorescent signal generated by the coupling of a fluorogenic dye molecule and a quencher moiety to the same or different oligonucleotide substrates. Examples of commonly used probes used in kPCR and kRT-PCR include the following probes: TAQMAN® probes (Applied Biosystems, Foster City, CA), Molecular Beacons probes (PHRI, Neward, N.J.), SCORPION® probes (DXS Ltd, Manchester, UK), and SYBR® Green probes (Invitrogen, Carlsbad, CA). Briefly, TAQMAN® probes, Molecular Beacons, and SCORPION® probes each have a fluorescent reporter dye (also called a "fluor") attached to the 5′ end of the probes and a quencher moiety coupled to the 3′ end of the probes. In the unhybridized state, the proximity of the fluor and the quench molecules prevents the detection of fluorescent signal from the probe. By contrast, during PCR, when the polymerase replicates a template on which a probe is bound, the 5′-nuclease activity of the polymerase cleaves the probe thus increasing fluorescence with each replication cycle. SYBR® Green probes binds double-stranded DNA and upon excitation emit light; thus as PCR product accumulates, fluorescence increases.

[0041] The terms "complementary" and "substantially complementary" refer to base pairing between nucleotides or nucleic acids, such as, for instance, between the two strands of a double-stranded DNA molecule or between an oligonucleotide primer and a primer binding site on a single-stranded nucleic acid to be sequenced or amplified. Complementary nucleotides are, generally, A and T (or A and U), and G and C. Within the context of the present invention, it is to be understood that the specific sequence lengths listed are illustrative and not limiting and that sequences covering the same map positions, but having slightly fewer or greater numbers of bases are deemed to be equivalents of the sequences and fall within the scope of the invention, provided they will hybridize to the same positions on the target as the listed sequences. Because it is understood that nucleic acids do not require complete complementarity in order to hybridize, the probe and primer sequences disclosed

herein may be modified to some extent without loss of utility as specific primers and probes. Generally, sequences having homology of 80% or more fall within the scope of the present invention.

[0042] As used herein, the term "cloning" is used to refer to "molecular cloning," which is a process that creates multiple copies of a nucleic acid sequence (also referred to herein as an "insert"), such as unique genes or selectable genetic markers, from a single copy of the insert. The cloning process typically occurs in a "cloning vector" (also referred to herein as "vector"), which is a DNA molecule, such as a plasmid or viral DNA chromosome, that is capable of replication in a suitable host cell. A "plasmid" is known in the art as a circular double-stranded DNA molecule that is obtained from a bacterial species. A cloning vector typically has one or more suitable sites for the insertion of the nucleic acid sequences. In a successful cloning, the cloning vector is introduced into the host cell and replication of the cloning vector in the host cell results in a transformed host cell, which expresses the nucleic acid sequences that were inserted into the cloning vector. Replication of the cloning vector in the host cell is typically initiated in via a "promoter," which is a regulatory region of DNA located upstream (towards the 5' region) of a gene, and which binds RNA polymerase and transcription factors to initiate RNA transcription. Using this procedure and as shown in FIG. 1, the cloning vector can be used as a template to produce an RNA internal control by routine transcription reaction or a DNA internal control by restriction digestion.

[0043] As explained in the Background section, when a non-competitive internal control is used in an amplification reaction, such as PCR, different primer sets are used for the internal control and for the target. The use of the non-competitive internal controls thus requires a PCR in which two reactions with different kinetics proceed simultaneously and the kinetics of each reaction are not influenced by competition for the primers.

[0044] An advantage of non-competitive internal controls over competitive internal controls is that because non-competitive internal controls are prepared with their own set of primers, they may be used for many different assays in the same laboratory. Another advantage of non-competitive internal controls is that they can be used for multiplex PCR assays. By contrast, competitive internal controls cannot be used with multiplex PCR assays in which several primer pairs are required. As is known to those of skill in the art, multiplex PCR has much usefulness for molecular diagnostics since multiple pathogens producing similar symptoms may be screened simultaneously in a single reaction.

[0045] The non-competitive internal controls of the present invention have at least two primer binding sites and at least one probe binding site. As non-competitive controls, the

internal controls of the present invention have unique cloning sequences that do not compete with the target nucleic acid sequences. The internal controls are independently designed from the genomes of the organisms *Methanobacterium thermoautrophicum* (MET) *Zea Mays* (Corn). Nucleic acids isolated from the organisms are constructed into a plasmid with a cloning vector.

[0046] Procedures for cloning nucleic acids are known to those of skill in the art. FIG. 1 shows an exemplary procedure to clone the internal control nucleic acids of the present invention; the procedure set forth in FIG. 1 was used to generate the non-competitive internal controls described in the Examples. As shown in FIG. 1, the isolated genomic DNA is digested with XhoI and SpeI restriction enzymes and the resulting DNA insert is amplified using PCR and purified. Separately, a vector fragment is prepared from the TOPO® Cloning Vector (Invitrogen, Carlsbad, California), which is digested with XhoI and SpeI restriction enzymes to form a vector fragment with XhoI and SpeI sticky ends, which is subsequently purified. The DNA insert and the vector fragment are then ligated by matching the XhoI and SpeI sticky ends to form a plasmid that includes the genomic DNA insert, the vector fragment, and a T7 promoter sequence. The T7 promoter sequence will typically be either a 20-mer T7 promoter sequence 5'-TAA TAC GAG TCA CTA TAG GG-3' (SEQ ID NO. 1) or a 21-mer T7 promoter sequence 5'-TAA TAC GAG TCA CTA TAG GGA-3' (SEQ ID NO. 2). The internal control DNA sequences of the present invention are generated by digesting the plasmid with XhoI or SpeI. The internal control RNA sequences are obtained by transcribing the DNA under conditions known to those of skill in the art.

[0047] Modifications to the procedures set forth in FIG. 1, such as for example, replacing the T7 promoter with other promoters known in the art, such as the T3 and SP6 promoters, or flanking the MET or Corn inserts with more than one promoter are within the skill level of those in the art. Similarly, it would be within the skill level of one in the art to modify the method set forth herein by replacing the XhoI and SpeI restriction binding sites with binding sites for other suitable restriction enzymes.

[0048] The DNA internal controls of the present invention have utility in DNA nucleic acid tests (DATs), including without limitation: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (GC), and Hepatitis B virus (HBV).

[0049] The RNA internal controls of the present invention have utility in RNA nucleic acid tests (NATs), including without limitation: Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

[0050] In practice, it is to be understood that the internal controls of the present invention are included in the same reaction mix as the sample that is being targeted. In one embodiment of the invention, the internal controls are introduced at the step of virus lysis and consequently, can be used to monitor the RNA or DNA target capture or release at the sample preparation step and/or to monitor the target amplification and detection during real time PCR.

[0051] INTERNAL CONTROL FRAGMENTS AND PRIMER AND PROBE SETS:

[0052] A sequence listing describes the (i) DNA insert during cloning; (ii) resulting complete double-stranded DNA sequence based on purification following restriction enzyme digest; and (iii) sequence of the single-stranded RNA generated from the T7 promoter with attached vector sequences.

[0053] MET NUCLEIC ACID FRAGMENTS

[0054] Table 1 shows the sequences of the forward (FP) and reverse (RP) primers that are used to extract nucleic acid fragments from the *M. thermoautrophicum* (MET) genome, which are used to clone the MET internal controls (MET IC) of the present invention. The primers are designed with restriction enzymes binding sites (highlighted in bold) and target specific binding sites (underlined). As shown therein, the forward primer is designed with an XhoI restriction enzyme sequence (C/TCGAG) and the reverse primer is designed with a SpeI restriction enzyme sequence (A/CTAGT).

TABLE 1

Fragment Primers	Sequence 5 -3	Strands
MET IC FP	AGTAGTC**TCGAG**CATGTGCAGGGATCCTGACA (SEQ ID. NO. 3)	(+)
MET IC RP	TCGTCGA**CTAGT**TCACCGAGCACCTCCTTCAGGCT (SEQ ID NO. 4)	(-)

[0055] As indicated above, the internal controls of the present invention include at least two primer binding sites and at least one probe binding site. Tables 2 to 8 set forth various forward and reverse amplification primer sequences and detection probe binding sequences that can be used to generate MET IC Amplicons, which are also provided in the tables. The

amplicons set forth in Tables 2 to 8 can be used to prepare non-competitive controls for use in nucleic acid diagnostic tests for the following disease states: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (GC), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

TABLE 2

Set 1	**Sequence 5 -3**	**Strands**
MET- FW01	GCCGCCATAAGAGCCATAGAA (SEQ ID NO. 5)	(+)
MET- RV01	GGGTAATTTGTCTCTGGCTTTGA (SEQ ID NO. 6)	(-)
MET-P01	CGCCCTTTGATATCTGCTCCGCAG (SEQ ID NO. 7)	(+)
MET IC Amplicon (96 pbs)	1 GCCGCCATAA GAGCCATAGA GGAGGTTGAG 31 GGTGTTGTGA CGCCCTTTGA TATCTGCTCC 61 GCAGCATCAA AGCCAGAGAC AAATTACCC (SEQ ID No. 8)	

TABLE 3

Set 2	**Sequence 5 -3**	**Strands**
MET-FW02	AGAGGAGGTTGAGGGTGTTGTG (SEQ ID NO. 9)	(+)
MET-RV01	GGGTAATTTGTCTCTGGCTTTGA (SEQ ID NO. 6)	(-)
MET-P02	CGCCCTTTGATATCTGCTCCGCAG (SEQ ID NO. 7)	(+)
Set 2 Amplicon (80 bps)	1 AGAGCCATAG AGGAGGTTGA GGGTGTTGTG 31 ACGCCCTTTG ATATCTGCTC CGCAGCATCA 61 AAGCCAGAGA CAAATTACCC C (SEQ ID NO. 10)	

TABLE 4

Set 3	**Sequence 5 -3**	**Strands**
MET-FW03	TAGAGGAGGTTGAGGGTGTTGTG (SEQ ID NO. 11)	(+)
MET-RV01	GGGTAATTTGTCTCTGGCTTTGA	(-)

TABLE 4		
Set 3	**Sequence 5 -3**	**Strands**
	(SEQ ID NO. 6)	
MET-P02	CGCCCTTTCATATCTGCTCCGCAG (SEQ ID NO. 7)	(+)
Set 3 Amplicon (81 bps)	1 TAGAGCCATA GAGGAGGTTG AGGGTGTTGT 31 GACGCCCTTT GATATCTGCT CCGCAGCATC 61 AAAGCCAGAG ACAAATTACC C (SEQ ID NO. 12)	

TABLE 5		
Set 4	**Sequence 5 -3**	**Strands**
MET-FW04	ATAGAGGAGGTTGAGGGTGTTGTG (SEQ ID NO. 13)	(+)
MET-RV01	GGGTAATTTGTCTCTGGCTTTGA (SEQ ID NO. 6)	(-)
MET-P02	CGCCCTTTCATATCTGCTCCGCAG (SEQ ID NO. 7)	(+)
Set 4 Amplicon (82 bps)	1 ATAGAGCCAT AGAGGAGGTT GAGGGTGTTG 31 TGACGCCCTT TGATATCTGC TCCGCAGCAT 61 CAAAGCCAGA GACAAATTAC CC (SEQ ID NO. 14)	

TABLE 6		
Set 5	**Sequence 5 -3**	**Strands**
MET-FW05	ATAGAGGAGGTTGAGGGTGTTGTG (SEQ ID NO. 15)	(+)
MET-RV05	CCTTCAGGCTCGGGCAGTA (SEQ ID NO. 16)	(-)
MET-P05	CCCTTTGAGATCTGCTCCGCA (SEQ ID NO. 17)	(+)
Set 5 Amplicon (121 bps)	1 ATAGAGGAGG TTGAGGGTGT TGTGACGCCC 31 TTTGAGAGCT GCTCCGCAGC ATCAAAGCCA 61 GAGACAAATT ACCCCTGGAT AGGCCCCACC 91 ACGAACCACC CCTACTGCCC GAGCCTGAAG 121 G (SEQ ID NO. 18)	

TABLE 7		
Set 6	**Sequence 5 -3**	**Strands**

TABLE 7		
Set 6	**Sequence 5 -3**	**Strands**
MET-FW06	AATTGGGCCCTCTAGATGCA (SEQ ID NO. 19)	(+)
MET-RV06	GATATCAAAGGGCGTCACAACA (SEQ ID NO. 20)	(-)
MET-P06	CAGGGCCGCCATAAGAGCCATAG (SEQ ID NO. 21)	(+)
Set 6 Amplicon (122 bps) (Vector sequence is highlighted in bold underlining)	1 **AATTGGGCCC** **TCTAGATGCA** **TG**CTCGAGCA 31 TGTGCAGGGA TCCTGACACG GTACTGGAGG 61 CAGGCAGGGC CGCCATAAGA GCCATAGAGG 91 AGGTTGAGGG TGTTGTGACG CCCTTTGAGA 121 TC (SEQ ID NO. 22)	

TABLE 8		
Set 7	**Sequence 5 -3**	**Strands**
MET-FW07	TTGTGACGCCCTTTGATATCTG (SEQ ID NO. 23)	(+)
MET-RV05	CCTTCAGGCTCGGGCAGTA (SEQ ID NO. 16)	(-)
METP07a	CTGGATAGGCCCCACCACGAACC (SEQ ID NO. 24)	(+)
METP07b	TCCGCAGCATCAAAGCCAGAGACA (SEQ ID NO. 25)	
Set 7 Amplicon (102 bps)	1 TTGTGACGCC CTTTGATATC TGCTCCGCAG 31 CATCAAAGCC AGAGACAAAT TACCCCTGGA 61 TAGGCCCCAC CACGAACCAC CCCTACTGCC 91 CGAGCCTGAA GG (SEQ ID NO. 26)	

[0056] CORN NUCLEIC ACID FRAGMENTS

[0057] Table 9 shows the sequences of the forward (FP) and reverse (RP) primers that are used to extract nucleic acid fragments from the *Z. mays* (Corn) genome, which are used to clone the Corn internal controls (Corn IC) of the present invention. The primers are designed with restriction enzymes binding sites (highlighted in bold) and target specific binding sites (underlined). As shown therein, the forward primer is designed with an XhoI restriction enzyme sequence (C/TCGAG) and the reverse primer is designed with a SpeI restriction enzyme sequence (A/CTAGT).

TABLE 9		
Fragment Primers	**Sequence 5 -3**	**Strands**
Corn 1 IC FP	AGTAGTC**TCGAG**TAAATAGCCCTCACCCACCAAC (SEQ ID. NO. 27)	(+)
Corn 1 IC RP	TCGTCGA**CTAGT**CCGAGAGCGCAGGCTTC (SEQ ID NO. 28)	(-)

[0058] As indicated above, the internal controls of the present invention include at least two primer binding sites and at least one probe binding site. Tables 10 to 12 set forth various forward and reverse amplification primer sequences and detection probe binding sequences that can be used to generate Corn IC Amplicons which are also provided in the tables. The amplicons set forth in Tables 10 to 12 can be used to prepare non-competitive controls for use in nucleic acid diagnostic tests for the following disease states: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), *Chlamydia trachomatis* (CT), *Neisseria gonorrhea* (GC), Hepatitis B virus (HBV), Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

TABLE 10		
Set 1	**Sequence 5 -3**	**Strands**
Corn1 IC FP1	AATAGCCCTCACCCACCAACT (SEQ ID NO. 29)	(+)
Corn1 IC RP1	TCCAACGGCTCTGTGTCAGA (SEQ ID NO. 30)	(-)
Corn1 IC Probe 1	CCGTTACAGGCAAGTTACTGCG (SEQ ID NO. 31)	(+)
Corn IC Amplicon (150 pbs)	1 AATAGCCCTC ACCCACCAAC TAGCCGTTAC 31 AGGCAAGTTA CTGCGCGATG GCGCACCGGA 61 CAGTCCGGTG CGCCACCGGT GCGCCACCGG 91 TGCGCCACCG GTGCGCCAAC GGTCACTTNC 121 AACGGCTAGT TCTGACACAG AGCCGTTGGA (SEQ ID NO. 32)	

TABLE 11		
Set 2	**Sequence 5 -3**	**Strands**

TABLE 11		
Set 2	**Sequence 5 -3**	**Strands**
Corn1 IC FP2	AAATAGCCCTCACCCACCAACT (SEQ ID NO. 33)	(+)
Corn1 IC RP1	TCCAACGGCTCTGTGTCAGA (SEQ ID NO. 30)	(-)
Corn1 IC Probe 1	CCGTTACAGGCAAGTTACTGCG (SEQ ID NO. 31)	(+)
Set 2 Amplicon (151 pbs)	1 AAATAGCCCT CACCCACCAA CTAGCCGTTA 31 CAGGCAAGTT ACTGCGCGAT GGCGCACCGG 61 ACAGTCCGGT GCGCCACCGG TGCGCCACCG 91 GTGCGCCACC GGTGCGCCAA CGGTCACTTN 121 CAACGGCTAG TTCTGACACA GAGCCGTTGG 151 A (SEQ ID NO. 34)	

TABLE 12		
Set 3	**Sequence 5 -3**	**Strands**
Corn1 IC FP1	AATAGCCCTCACCCACCAACT (SEQ ID NO. 29)	(+)
Corn1 IC RP3	GTCCAACGGCTCTGTGTCAGA (SEQ ID NO. 35)	(-)
Corn1 IC Probe 1	CCGTTACAGGCAAGTTACTGCG (SEQ ID NO. 31)	(+)
Set 3 Amplicon (151 pbs)	1 AATAGCCCTC ACCCACCAAC TAGCCGTTAC 31 AGGCAAGTTA CTGCGCGATG GGTCACTTNC 61 CAGTCCGGTG CGCCACCGGT GCGCCACCGG 91 TGCGCCACCG GTGCGCCAAC GGTCACTTNC 121 AACGGCTAGT TCTGACACAG AGCCGTTGGA 151 C (SEQ ID NO. 36)	

[0059] It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the foregoing description as well as the examples that follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

[0060] All patents and publications mentioned herein are incorporated by reference in their entireties.

EXPERIMENTAL

[0061] The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make and use the compositions of the invention. The examples are intended as non-limiting examples of the invention. While efforts have been made to ensure accuracy with respect to variables such as amounts, temperature, etc., experimental error and deviations should be taken into account. Unless indicated otherwise, parts are parts by weight, temperature is degrees centigrade, and pressure is at or near atmospheric. All components were obtained commercially unless otherwise indicated.

[0062] GENERAL PROTOCOLS:

[0063] The following protocols, apparatus, and kits were used to carry out the following Examples.

Extraction of Genomic DNA: native target purified by manual Qiagen (Valencia, CA) sample preparation kit.

[0064] PCR Conditions and Apparatus: PCR was conducted on an MJ Research (Ramsey, MN) instrument using the following thermoprofile:

[0065] 95° 10 min – 1 step

[0066] 95° 15 sec

[0067] 60° 15 sec } repeated for 30 cycles

[0068] 68° 1 min

[0069] 72° 10 min

[0070] 4° overnight (until removed)

[0071] Purification of PCR Product: PCR product was purified using a Qiagen kit (Valencia, CA)

[0072] Cloning Procedure: Cloning was carried out using the procedure set forth in the product insert for the Invitrogen cloning protocol using a TOPO® Cloning vector (Carlsbad, CA).

[0073] Ligation Procedure: Ligation of DNA fragments to the TOPO® Cloning vector was carried out using the instructions for Invitrogen T4 ligase (Carlsbad, CA).

[0074] Purification of Vector Fragment: Purification of vector was carried out using Clontech NUCLEOSPIN® RNA Purification Kit (Mountain View, CA).

[0075] RNA Transcription Protocol: Transcription was carried out according to the instructions in the product insert for the Ambion T7 MEGASCRIPT® kit (Austin, TX).

Purification of the RNA was carried out according to the instructions in the Qiagen RNEASY® mini kit (Valencia, CA).

EXAMPLE 1

PREPARATION OF MET IC DNA INSERT SEQUENCE

[0076] Genomic DNA was extracted from a MET sample. The DNA insert was prepared by running a PCR on the genomic DNA with the fragment primers of Table 1. The following sequence is the sequence for the MET IC PCR Product (213 bp) (SEQ ID NO. 37). The XhoI and SpeI restriction enzyme sites are identified with bold underlining.

1	AGTAGTC**TCG**	**AG**CATGTGCA	GGGATCCTGA	CACGGTACTG
	TCATCAG**AGC**	**TC**GTACACGT	CCCTAGGACT	GTGCCATGAC
41	GAGGCAGGCA	GGGCCGCCAT	AAGAGCCATA	GAGGAGGTTG
	CTCCGTCCGT	CCCGGCGGTA	TTCTCGGTAT	CTCCTCCAAC
81	AGGGTGTTGT	GACGCCCTTT	GATATCTGCT	CCGCAGCATC
	TCCCACAACA	CTGCGGGAAA	CTATAGACGA	GGCGTCGTAG
121	AAAGCCAGAG	ACAAATTACC	CCTGGATAGG	CCCCACCACG
	TTTCGGTCTC	TGTTTAATGG	GGACCTATCC	GGGGTGGTGC
161	AACCACCCCT	ACTGCCCGAG	CCTGAAGGAG	GTGCTCGGTG
	TTGGTGGGGA	TGACGGGCTC	GGACTTCCTC	CACGAGCCAC
201	AA**CTAGT**CGA	CGA		
	TT**GATCA**GCT	GCT		

EXAMPLE 2

PURIFIED MET IC DNA INSERT SEQUENCE

[0077] The following sequence is the purified 195 bp dsDNA sequence following restriction enzyme digestion at the sites identified above (SEQ ID NO. 38):

1	TCGAGCATGT	GCAGGGATCC	TGACACGGTA	CTGGAGGCAG
41	GCAGGGCCGC	CATAAGAGCC	ATAGAGGAGG	TTGAGGGTGT
81	TGTGACGCCC	TTTGATATCT	GCTCCGCAGC	ATCAAAGCCA
121	GAGACAAATT	ACCCCTGGAT	AGGCCCCACC	ACGAACCACC
161	CCTACTGCCC	GAGCCTGAAG	GAGGTGCTCG	GTGAA

EXAMPLE 3

MET IC DNA TRANSCRIPT SEQUENCE

[0078] A plasmid was prepared by ligating the purified MET IC DNA insert sequence of Example 2 to a purified vector fragment and adding a T7 promoter sequence. The purified vector fragment was isolated from a TOPO® Cloning vector (Invitrogen, Carlsbad, California) via digestion with the restriction enzymes XhoI and SpeI. The plasmid was formed by matching the XhoI and SpeI sticky ends of the DNA insert and the vector. FIG. 1 shows a schematic of the cloning process.

[0079] The resultant plasmid was linearized with Xho1 and SpeI to generate the following 247 bp MET IC DNA transcript sequence. The vector sequences are highlighted with bold underlining (SEQ ID NO. 39):

1	**GGGCGAATTG**	**GGCCCTCTAG**	**ATGCATG**CTC	GAGCATGTGC
41	AGGGATCCTG	ACACGGTACT	GGAGGCAGGC	AGGGCCGCCA
81	TAAGAGCCAT	AGAGGAGGTT	GAGGGTGTTG	TGACGCCCTT
121	TGATATCTGC	TCCGCAGCAT	CAAAGCCAGA	GACAAATTAC
161	CCCTGGATAG	GCCCCACCAC	GAACCACCCC	TACTGCCCGA
201	GCCTGAAGGA	GGTGCTCGGT	GAACTAGT**GG**	**ATCCGAGCTC**
241	**GGTACCA**			

EXAMPLE 4

MET IC RNA TRANSCRIPT SEQUENCE

[0080] The following sequence is the 247 bp MET IC RNA transcript sequence prepared from the DNA sequence of Example 3. The vector sequences are highlighted with bold underlining (SEQ ID NO. 40):

1	**GGGCGAAUUG**	**GGCCCUCUAG**	**AUGCAUG**CUC	GAGCAUGUGC
41	AGGGAUCCUG	ACACGGUACU	GGAGGCAGGC	AGGGCCGCCA
81	UAAGAGCCAU	AGAGGAGGUU	GAGGGUGUUG	UGACGCCCUU
121	UGAUAUCUGC	UCCGCAGCAU	CAAAGCCAGA	GACAAAUUAC
161	CCCUGGAUAG	GCCCCACCAC	GAACCACCCC	UACUGCCCGA
201	GCCUGAAGGA	GGUGCUCGGU	GAACUAGU**GG**	**AUCCGAGCUC**
241	**GGUACCA**			

EXAMPLE 5

PREPARATION OF CORN IC INSERT SEQUENCE

[0081] Genomic DNA was extracted from a *Z. mays* (Corn) sample. The DNA insert was prepared by running a PCR on the genomic DNA with the fragment primers of Table 9. The following sequence is the sequence for the Corn IC PCR Product (284 bp) (SEQ ID NO. 41). The XhoI and SpeI restriction enzyme sites are identified with bold underlining.

```
1    AGTAGTCTCG    AGTAAATAGC    CCTCACCCAC    CAACTAGCCG
     TCATCAGAGC    TCATTTATCG    GGAGTGGGTG    GTTGATCGGC

41   TTACAGGCAA    GTTACTGCGC    GATGGCGCAC    CGGACAGTCC
     AATGTCCGTT    CAATGACGCG    CTACCGCGTG    GCCTGTCAGG

81   GGTGCGCCAC    CGGTGCGCCA    CCGGTGCGCC    ACCGGTGCGC
     CCACGCGGTC    GCCACGCGGT    GGCCACGCGG    TGGCCACGCG

121  CAACGGTCAC    TTNCAACGGC    TAGTTCTGAC    ACAGAGCCGT
     GTTGCCAGTG    AANGTTGCCG    ATCAAGACTG    TGTCTCGGCA

161  TGGACTCATG    ACGCACCGGA    CAGTGAATAG    TTCACTGTCC
     ACCTGAGTAC    TGCGTGGCCT    GTCACTTATC    AAGTGACAGG

201  GGTGCACACC    GGACAGTCCG    GTGCGGTGTC    CGGTGTGCCA
     CCACGTGTGG    CCTGTCAGGC    CACGCCACAG    GCCACACGGT

241  CTAAAATTCA    TCTCCGAAGC    CTGCGCTCTC    GGACTAGTCG
     GATTTTAAGT    AGAGGCTTCG    GACGCGAGAG    CCTGATCAGC

281  ACGA
     TGCT
```

EXAMPLE 6

PURIFIED CORN IC DNA INSERT SEQUENCE

[0082] The following sequence is the purified 266 bp dsDNA sequence following restriction enzyme digestion at the sites identified above (SEQ ID NO. 42):

```
1    TCGAGTAAAT    AGCCCTCACC    CACCAACTAG    CCGTTACAGG

41   CAAGTTACTG    CGVGATGGCG    CACCGGACAG    TCCGGTGCGC

81   CACCGGTGCG    CCACCGGTGC    GCCACCGGTG    CGCCAACGGT

121  CACTTCCAAC    GGCTAGTTCT    GACACAGAGC    CGTTGGACTC

161  ATGACGCACC    GGACAGTGAA    TAGTTCACTG    TCCGGTGCAC

201  ACCGGACAGT    CCGGTGCGGT    GTCCGGTGTG    CCACTAAAAT

241  TCATCTCCGA    AGCCTGCGCT    CTCGGA
```

EXAMPLE 7

CORN IC DNA TRANSCRIPT SEQUENCE

[0083] A plasmid was prepared by ligating the purified MET Corn IC DNA insert sequence of Example 6 to a purified vector fragment with sticky end restriction sites and a T7 promoter sequence. The purified vector fragment was isolated from a TOPO® Cloning vector (Invitrogen, Carlsbad, California) via digestion with the restriction enzymes XhoI and SpeI. The plasmid was formed by matching the XhoI and SpeI sticky ends of the DNA insert and the vector. FIG 1 shows a schematic of the cloning process.

[0084] The resultant plasmid was linearized with Xho1 and SpeI to generate the following 318 bp Corn IC DNA transcript sequence. The vector sequences are highlighted with bold underlining (SEQ ID NO. 43):

1	**GGGCGAATTG**	**GGCCCTCTAG**	**ATGCATG**CTC	GAGTAAATAG
41	CCCTCACCCA	CCAACTAGCC	GTTACAGGCA	AGTTACTGCG
81	CGATGGCGCA	CCGGACAGTC	CGGTGCGCCA	CCGGTGCGCC
121	ACCGGTGCGC	CACCGGTGCG	CCAACGGTCA	CTTNCAACGG
161	CTAGTTCTGA	CACAGAGCCG	TTGGACTCAT	GACGCACCGG
201	ACAGTGAATA	GTTCACTGTC	CGGTGCACAC	CGGACAGTCC
241	GGTGCGGTGT	CCGGTGTGCC	ACTAAAATTC	ATCTCCGAAG
281	CCTGCGCTCT	CGGACTAGT**G**	**CATCCGAGCT**	**CGGTACCA**

EXAMPLE 8

CORN IC RNA TRANSCRIPT SEQUENCE

[0085] The following sequence is the 318 bp Corn IC RNA transcript sequence prepared from the DNA sequence of Example 7. The vector sequences are highlighted with bold underlining (SEQ ID NO. 44):

1	**GGGCGAAUUG**	**GGCCCUCUAG**	**AUGCAUG**CUC	GAGUAAAUAG
41	CCCUCACCCA	CCAACUAGCC	GUUACAGGCA	AGUUACUGCG
81	CGAUGGCGCA	CCGGACAGUC	CGGUGCGCCA	CCGGUGCGCC
121	ACCGGUGCGC	CACCGGUGCG	CCAACGGUCA	CUUNCAACGG
161	CUAGUUCUGA	CACAGAGCCG	UUGGACUCAU	GACGCACCGG
201	ACAGUGAAUA	GUUCACUGUC	CGGUGCACAC	CGGACAGUCC

241	GGUGCGGUGU	CCGGUGUGCC	ACUAAAAUUC	AUCUCCGAAG
281	CCUGCGCUCU	CGGACUAGU**G**	**CAUCCGAGCU**	**CGGUACCA**

EXPERIMENT 9

USE OF MET IC IN CT, GC, AND HCV ASSAYS

[0086] Independent RT-PCR assays were carried out with TAQMAN® probes (Applied Biosystems, Foster City, CA) for CT, GC, and HCV, respectively. Each assay included the target nucleic acid (DNA for CT and GC and RNA for HCV) and the MET IC of the present invention in a single well. Figures 2, 3, and 4 show the results of the amplification assays (cycle number versus delta Rn). Rn, the normalized reporter signal, is the fluorescence signal of the reporter dye divided by the fluorescence signal of the internal reference dye. Delta Rn (dRn) is determined by the formula $R^{n+} - R^{n-}$, where R^{n+} is the Rn value for a reaction involving all components and R^{n-} is the value for an unreacted sample. In each graph, the curve on the left represents amplification of the target and the curve on the right represents amplification of the IC.

WE CLAIM:

1. A non-competitive internal control for use in nucleic acid tests (NATs), comprising a nucleic acid obtained from an organism selected from *Methanobacterium thermoautrophicum* (MET) or *Zea mays*.

2. The non-competitive internal control of claim 1, wherein the nucleic acid is DNA.

3. The non-competitive internal control of claim 2, wherein the NATs are selected from the group consisting of: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), Hepatitis B virus (HBV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhea* (GC).

4. The non-competitive internal control of claim 1, wherein the nucleic acid is RNA.

5. The non-competitive internal control of claim 4, wherein the diagnostic NATs are selected from the group consisting of Hepatitis C virus (HCV), Human Immunodeficiency Virus 1 (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

6. The non-competitive internal control of claim 1, wherein the nucleic acid comprises at least at least two primer binding sites and at least one probe binding site.

7. A method of preparing a non-competitive internal control for use in nucleic acid tests (NATs), comprising the steps of:

(a) extracting genomic DNA from *Methanobacterium thermoautrophicum* (MET);

(b) generating an amplicon from the genomic DNA of step (a) using forward and reverse primers having at least two restriction enzyme sites and a target specific sequence;

(c) generating a plasmid by ligating the amplicon of step (b) with a vector sequence having a promoter sequence and restriction enzyme sites that are identical to the restriction enzyme sites of the amplicon;

(d) digesting the plasmid with restriction enzymes corresponding to the restriction enzyme sites of steps (b) and (c) to generate MET internal control DNA.

8. The method of claim 7, further comprising the step of:

(e) preparing MET internal control RNA from the DNA of step (e).

9. The method of claim 7, wherein the at least two restriction enzyme sites of steps (b) and (d) correspond to the sequences of restriction enzymes XhoI and SpeI.

10. The method of claim 7, wherein the forward primer of step (b) has the sequence of SEQ ID. NO. 3 and the reverse primer of step (b) has the sequence of SEQ ID. NO. 4.

11. The method of claim 7, wherein the promoter sequence of step (c) is a T7 promoter sequence.

12. The method of claim 7, wherein the MET internal control DNA is used as an internal control in DNA nucleic acid diagnostic tests for the following disease states: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), RSV B, human metapneumovirus (hMPV), Hepatitis B virus (HBV), *Chlamydia trachomatis* (CT), and *Neisseria gonorrhea* (GC).

13. The method of claim 8, wherein the MET internal control RNA is used as an internal control in RNA nucleic acid diagnostic tests for the following disease states: Hepatitis C virus (HCV), Human Immunodeficiency Virus I (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

14. A method of preparing a non-competitive internal control for use in nucleic acid tests (NATs) comprising the steps of:

(a) extracting genomic DNA from *Zea Mays* (Corn);

(b) generating an amplicon from the genomic DNA of step (a) using forward and reverse primers having at least two restriction enzyme sites and a target specific sequence;

(c) generating a plasmid by ligating the amplicon of step (c) with a vector sequence having a promoter sequence and restriction enzyme sites that are identical to the restriction enzyme sites of the amplicon;

(d) digesting the plasmid with restriction enzymes corresponding to the restriction enzyme sites of steps (b) and (c) to generate Corn internal control DNA.

15. The method of claim 14, further comprising the step of:

(e) preparing Corn internal control RNA from the DNA of step (e).

16. The method of claim 14, wherein the at least two restriction enzyme sites of steps (b) and (d) correspond to the sequences of restriction enzymes XhoI and SpeI.

17. The method of claim 14, wherein the promoter sequence of step (d) is a T7 promoter sequence.

18. The method of claim 14, wherein the Corn internal control DNA is used as an internal control in DNA nucleic acid diagnostic tests for the following disease states: Influenza A, Influenza B, parainfluenza viruses 1 to 4 (PIV-1 to PIV-4), respiratory syncytial virus type A (RSV A), respiratory syncytial virus type B (RSV B), human metapneumovirus (hMPV), *Chlamydia trachomatis, Neisseria gonorrhea*, and Hepatitis B virus (HBV).

19. The method of claim 15, wherein the Corn internal control RNA is used as an internal control in RNA nucleic acid diagnostic tests for the following disease states: Hepatitis C virus (HCV), Human Immunodeficiency Virus I (HIV-1), and Severe Acute Respiratory Syndrome (SARS).

20. The method of claim 14, wherein the forward primer of step (b) has the sequence of SEQ ID NO. 27 and the reverse primer of step (b) has the sequence of SEQ ID NO. 28.

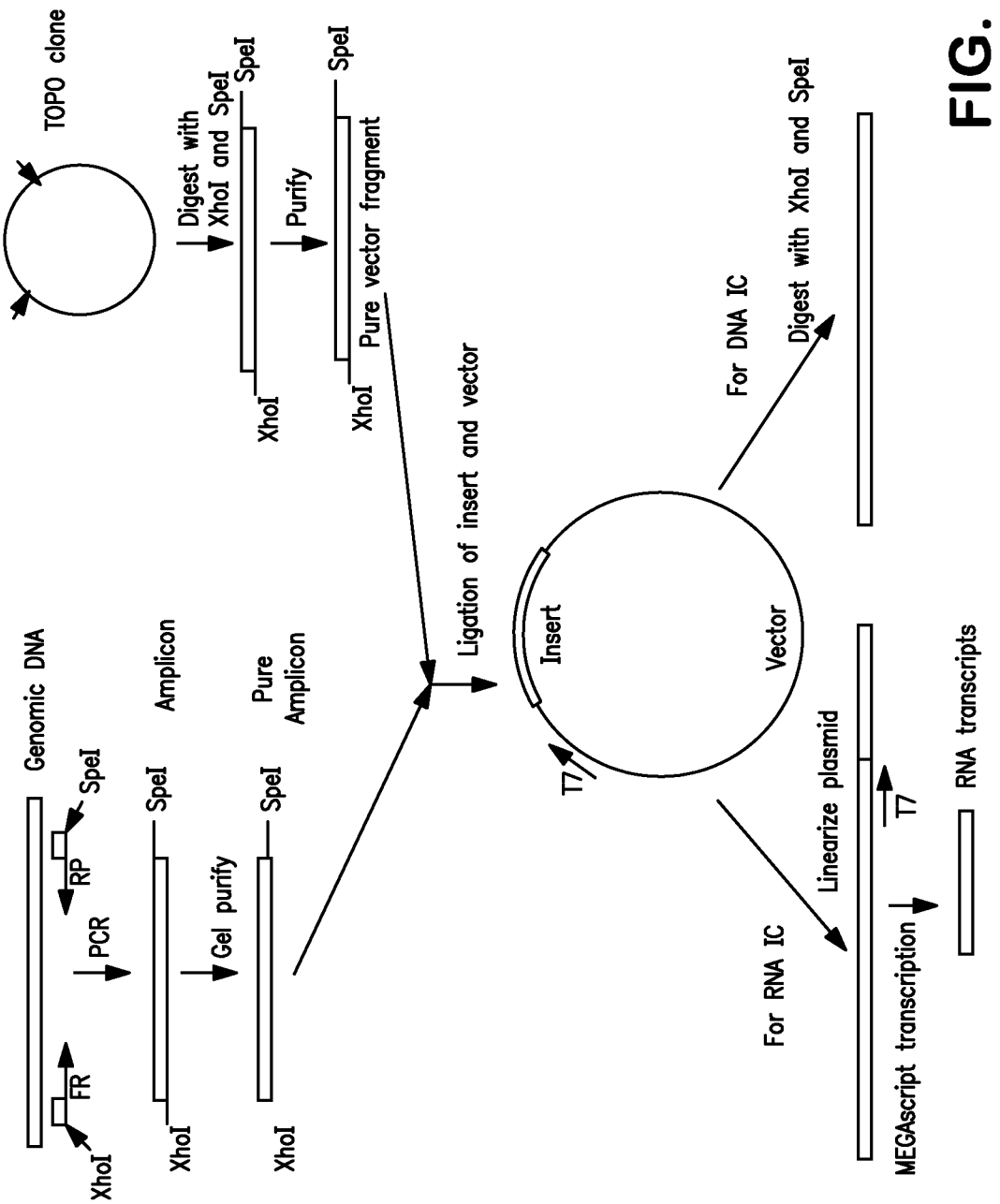
TOPO clone
Digest with XhoI and SpeI
SpeI
XhoI
Purify
SpeI
XhoI
Pure vector fragment
Genomic DNA
SpeI
RP
PCR
FR
XhoI
SpeI
Amplicon
Gel purify
XhoI
SpeI
Pure Amplicon
XhoI
Ligation of insert and vector
Insert
Vector
T7
For DNA IC
Digest with XhoI and SpeI
For RNA IC
Linearize plasmid
T7
MEGAscript transcription
RNA transcripts

FIG. 1

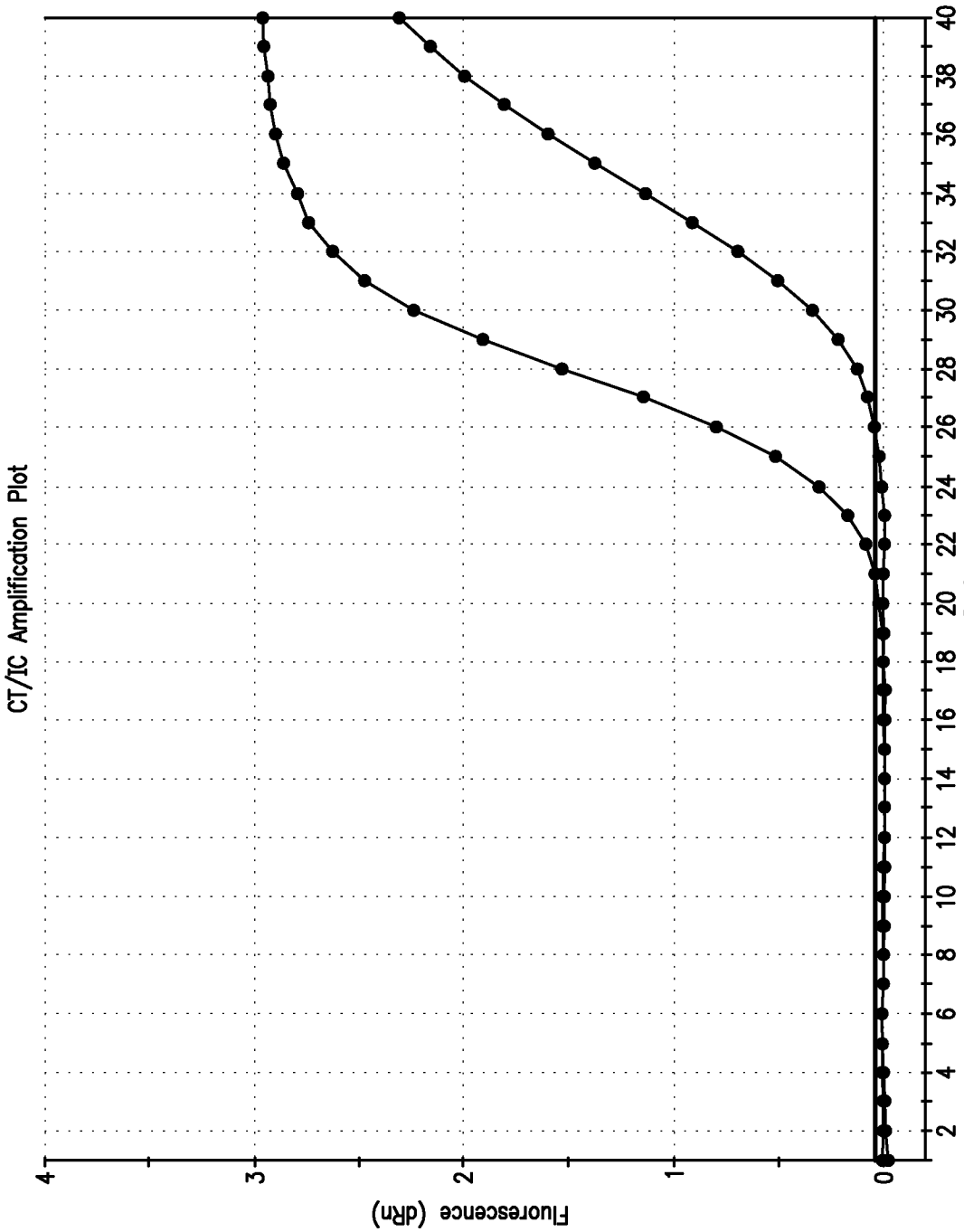
CT/IC Amplification Plot
Fluorescence (dRn)
Cycles
0
1
2
3
4
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40

FIG. 2

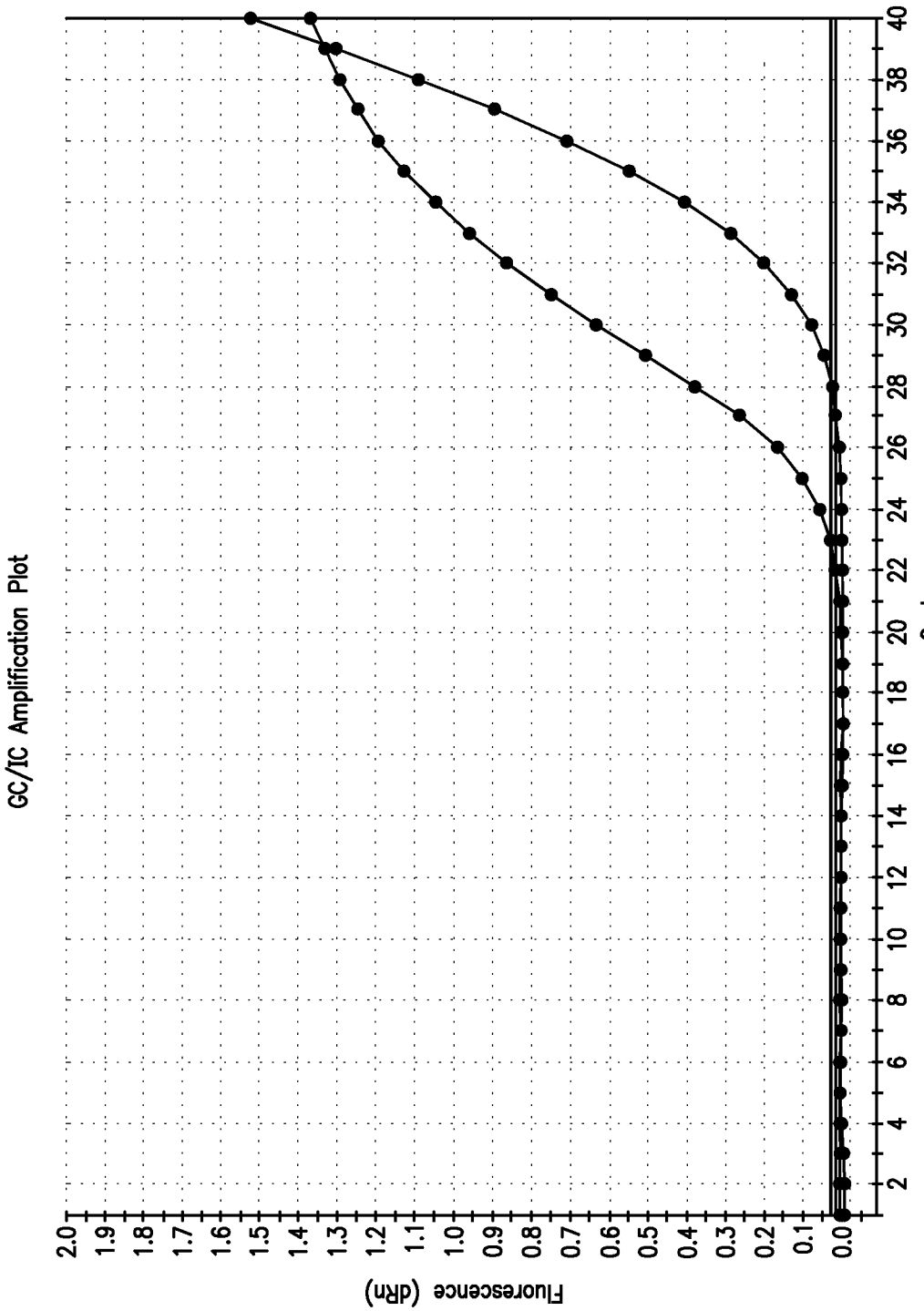
GC/IC Amplification Plot
Fluorescence (dRn)
Cycles
2.0
1.9
1.8
1.7
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40

FIG. 3

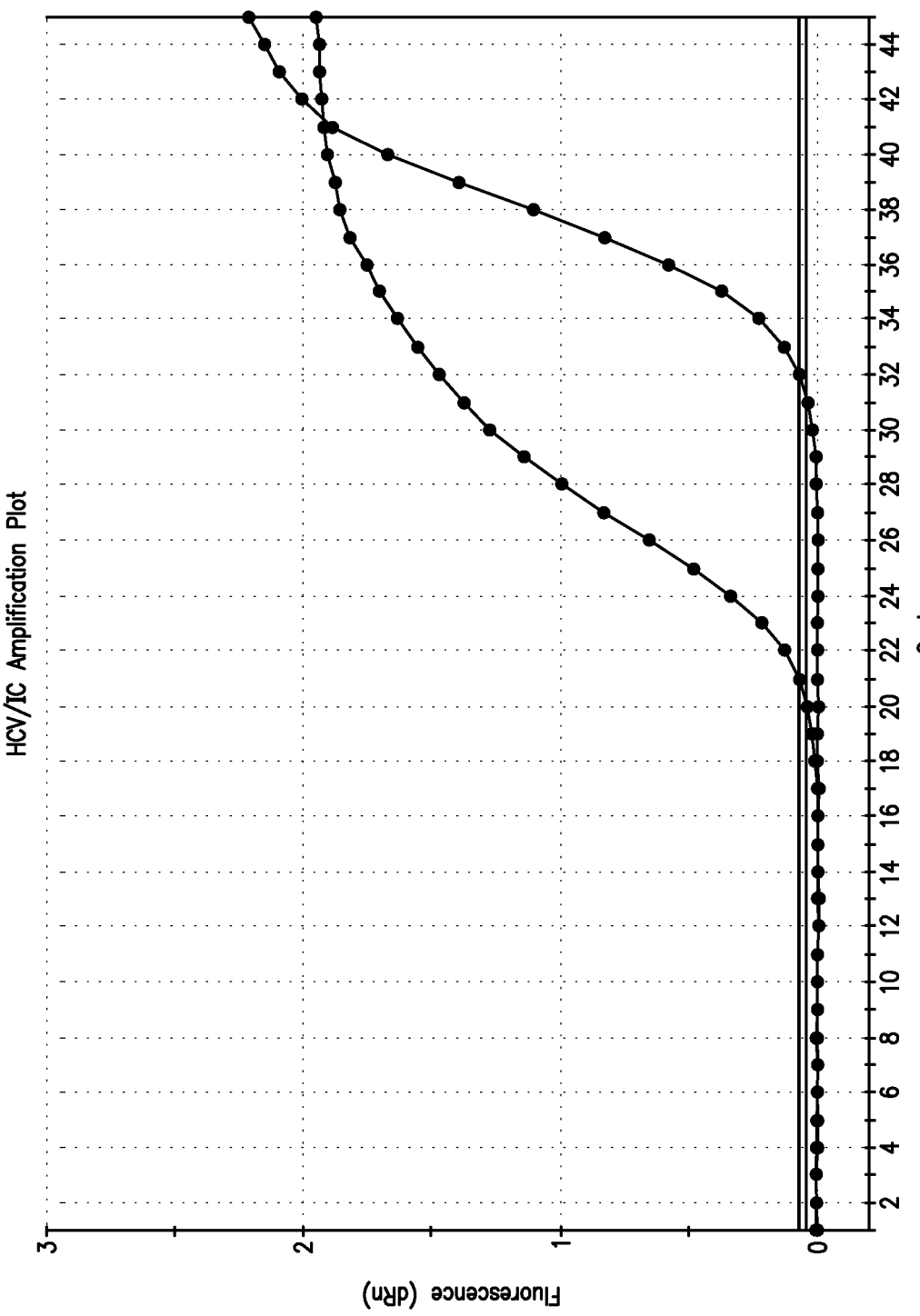
HCV/IC Amplification Plot
Fluorescence (dRn)
Cycles
0
1
2
3
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
32
34
36
38
40
42
44

FIG. 4

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/37593

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/68 (2009.01)
USPC - 435/6
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)
USPC - 435/6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 435/91.2; 536/23.1; 702/85 (text search, see terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,EPAB,JPAB); Google/Scholar; PubMed (text search, see terms below)
Search terms: Non-competitive, internal control, construct, prepare, HIV, hepatitis, influenza, SARS, chlamydia, Neisseria, methanobacterium, zea, restriction, enzyme, site, clone, amplify, primer, PCR.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y --- A	US 2001/0029014 A1 (BEUCKELEER) 11 October 2001 (11.10.2001); para [0006], [0029], [0037], [0070].	1, 2, 4, 6 --- 3, 5, 14-19 --- 20
Y	US 2006/0257860 A1 (MARLOWE et al.) 16 November 2006 (16.11.2006); Abstract, para [0040].	3, 12, 18
Y	TANG et al. A realtime HIV-1 viral load assay for automated quantitation of HIV-1 RNA in genetically diverse group M subtypes A-H, group O and group N samples. Journal of Virological Methods, 2007, vol146, pp 236-245; Abstract, (page 238, paras 1, 3).	5, 13, 19
Y --- A	US 2006/0112453 A1 (SUN et al.) 25 May 2006 (25.05.2006); para [0082], [0144], [0145].	7-9, 11-19 --- 10, 20
Y --- A	SMITH et al. Complete genome sequence of Methanobacterium thermoautotrophicum deltaH: functional analysis and comparative genomics. Journal of Bacteriology, November 1997, vol 179, No 22, pp 7135-7155; Abstract.	7-9, 11-13 --- 10
A	NCBI submission BV217527. January 2005 [online]. [Retrieved on 2009.05.12]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nuccore/57215342>.	10

☒ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"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
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 May 2009 (12.05.2009)	24 JUL 2009
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/37593

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NCBI submission CD165028. September 2003 [online]. [Retrieved on 2009.05.12]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nucest/34701691>.	10
A	NCBI submission CC841652. July 2003 [online]. [Retrieved on 2009.05.12]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nucgss/33185367>.	20
A	NCBI submission FC052757. December 2007 [online]. [Retrieved on 2009.05.12]. Retrieved from the internet: <URL: http://www.ncbi.nlm.nih.gov/nucest/161693742>.	20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 09/37593

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item1.b 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. type of material
   - [x] a sequence listing
   - [ ] table(s) related to the sequence listing

   b. format of material
   - [x] on paper
   - [ ] in electronic form

   c. time of filing/furnishing
   - [x] contained in the international application as filed
   - [ ] filed together with the international application in electronic form
   - [ ] furnished subsequently to this Authority for the purposes of search

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

3. Additional comments: