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(54) **PRIMER AND PROBE DESIGN FOR EFFICIENT AMPLIFICATION AND DETECTION OF HCV 3' NON-TRANSLATING REGION**

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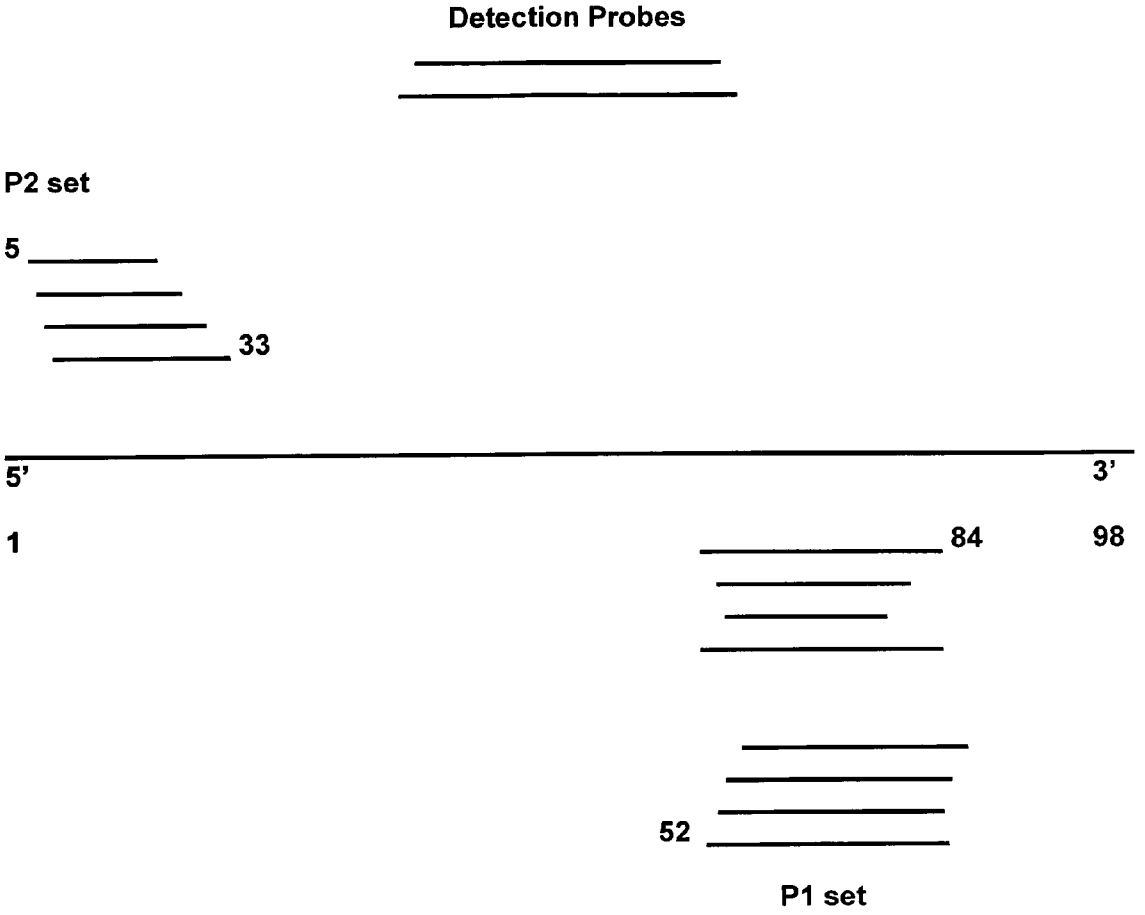
(57) **ABSTRACT**

The present invention provides selected oligonucleotides corresponding to portions of the 3' non-translating region of Hepatitis C virus. The invention also includes methods of detecting and of quantitating HCV nucleic acids in a sample using these oligonucleotides.



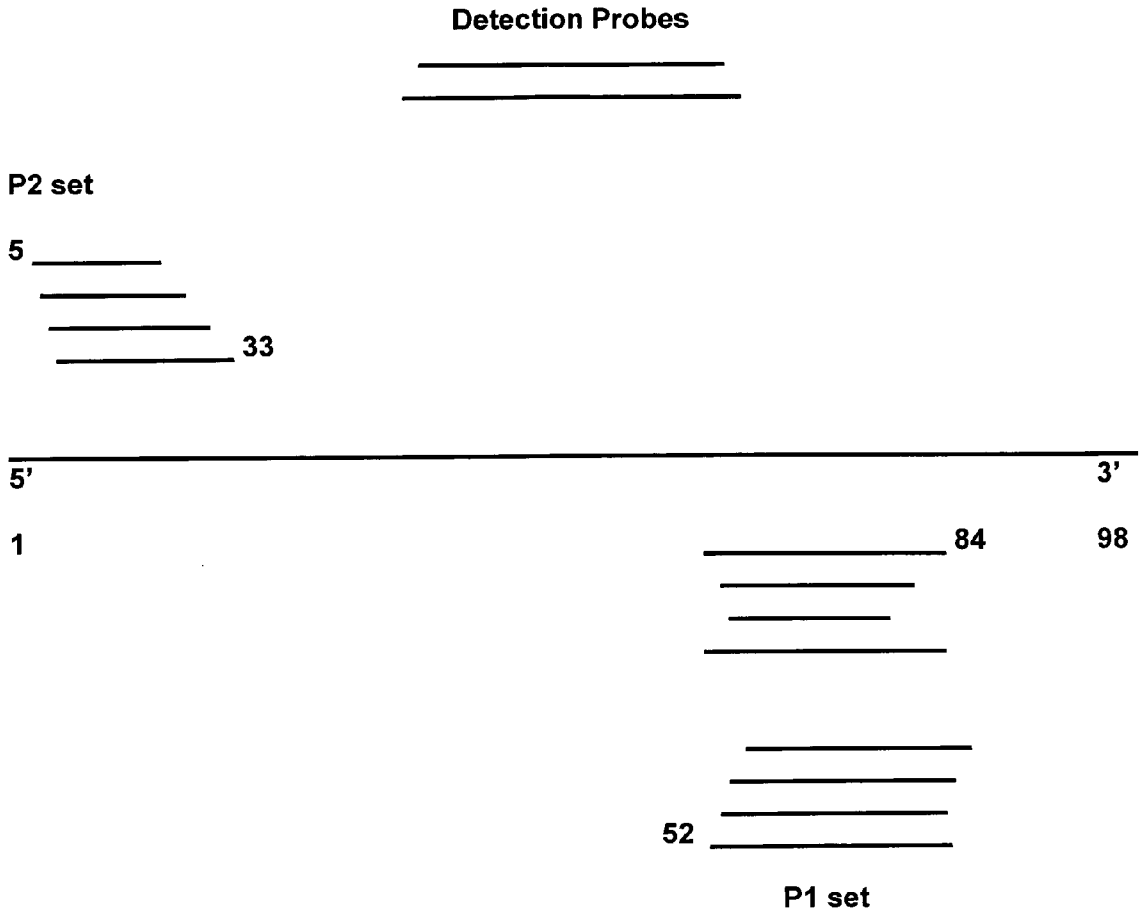


FIG. 1

PRIMER AND PROBE DESIGN FOR EFFICIENT AMPLIFICATION AND DETECTION OF HCV 3' NON-TRANSLATING REGION

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Patent Application No. 60/538,815 filed on Jan. 23, 2004, U.S. Provisional Patent Application No. 60/538,814 filed on Jan. 23, 2004, and U.S. Provisional Patent Application No. 60/538,816 filed on Jan. 23, 2004.

FIELD OF THE INVENTION

[0002] The present invention relates to oligonucleotides that bind to the 3' non-translating region (3'NTR) of Hepatitis C Virus (HCV), useful for the detection and/or quantification of HCV. Also provided are methods of nucleic acid assays useful in the detection, capture and amplification of HCV utilizing the 3'NTR as the target region.

BACKGROUND OF THE INVENTION

[0003] After the development of diagnostic tests for hepatitis A and hepatitis B viruses in the 1970s, an additional parenterally transmitted agent responsible for the majority of transfusion-associated non-A, non-B hepatitis cases was recognized. The identification of this agent turned out to be very difficult (Bartenschlager and Lohmann, *J. Gen. Virol.* 81: 1631-1648 (2000). With the advent of recombinant DNA, the genome of the virus that was termed hepatitis C virus (HCV) was cloned (Choo et al., *Science* 244: 359-362 (1989)). Its RNA has been difficult to study because biological materials are scarce and RNA replication is of low efficiency (Shi and Lai, Cell. Mol. Life Sci. 58: 1276-1295 (2001)).

[0004] Many general techniques are known for studying nucleic acids. When necessary, enzymatic amplification of nucleic acid sequences will enhance the ability to detect a desired nucleic acid sequence. Generally, the currently known amplification schemes can be broadly grouped into two classes based on whether the enzymatic amplification reactions are driven by continuous cycling of the temperature between the denaturation temperature, the primer annealing temperature, and the amplicon (product of enzymatic amplification of nucleic acid) synthesis temperature, or whether the temperature is kept constant throughout the enzymatic amplification process (isothermal amplification). Typical cycling nucleic acid amplification technologies (thermocycling) are polymerase chain reaction (PCR), and ligase chain reaction (LCR). Specific protocols for such reactions are discussed in, for example, Short Protocols in Molecular Biology, 2.sup.nd Edition, A Compendium of Methods from Current Protocols in Molecular Biology, (Eds. Ausubel et al., John Wiley & Sons, New York, 1992) chapter 15. Reactions which are isothermal include: transcription-mediated amplification (TMA), nucleic acid sequence-based amplification (NASBA), and strand displacement amplification (SDA).

[0005] U.S. Pat. Nos. 4,683,195 (Mullis); 4,965,188 (Mullis); and 4,683,202 (Mullis) describe a polymerase chain reaction (PCR) utilizes DNA polymerase, complementary primer molecules and repeated cycles of thermal reactions to exponentially replicate target nucleic acid molecules. Isothermal target amplification methods include transcription-based amplification methods, in which an RNA polymerase promoter sequence is incorporated into primer extension products at an early stage of the amplification (WO 89/01050), and further target sequence, or target complementary sequence, is amplified by transcription steps and digestion of an RNA strand in a DNA/RNA hybrid intermediate product. See, for example, U.S. Pat. Nos. 5,169,766 and 4,786,600. These methods include transcription mediated amplification (TMA), self-sustained sequence replication (3SR), Nucleic Acid Sequence Based Amplification (NASBA), and variations there of. See, for example, Guatelli et al. Proc. Natl. Acad. Sci. U.S.A. 87:1874-1878 (1990); U.S. Pat. Nos. 5,766,849 5,399,491; 5,480,784; 5,766,849; 5,466,586 (NASBA); 5,409,818(NASBA); 5,554,517(NASBA); 6,063,603(NASBA); 5,130,238 (NASBA); and 5,654,142 (TMA); and 5,130,238 (Malek et al.); 5,409,818 (Davey et al.); 5,654,142 (Kievits); and 6,312,928 (Van Gemen et al.) (nucleic acid sequence-based amplification (NASBA) techniques). U.S. Pat. No. 5,792,607 (Backman) describes amplification methods referred to as ligase chain reactions (LCR). U.S. Pat. Nos. 5,744,311 (Fraiser); 5,648,211 (Fraiser) and 5,631,147 (Lohman), describe isothermal amplification systems based on strand displacement amplification (SDA). Other approaches include Q.beta. replicase, strand displacement assay (SDA), transcription mediated iso CR cycling probe technology, nucleic acid sequence-based amplification (NASBA) and cascade rolling circle amplification (CRCA). Additional U.S. Patent documents which describe nucleic acid amplification include U.S. Pat. Nos. 4,876,187; 5,030,557; 5,399,491; 5,485,184; 5,554,517; 5,437,990; 5,399,491 and 5,554,516.

[0006] Nucleic acid hybridization techniques have been described for example, in Sambrook et al. Molecular Cloning A Laboratory Manual, 2nd Ed. Cold Spring Lab. Press, December 1989; U.S. Pat. Nos. 4,563,419 (Ranki) and 4,851,330 (Kohne) and in Dunn, et al., *Cell* 12, pp. 23-26 (1978) among many other publications.

[0007] Detection methods utilizing nucleic acids are also known. Nucleic acids are often labeled for various detection purposes. For example, methods described in U.S. Pat. Nos. 4,486,539 (Kourlisky); 4,411,955 (Ward); 4,882,269 (Schneider) and 4,213,893 (Carrico), illustrate preparation of labeled detection probes for detecting specific nucleic acid sequences. Furthermore, before or after exposing an extracted nucleic acid to a probe, the target nucleic acid can be immobilized by target-capture means, either directly or indirectly, using a "capture probe" bound to a substrate, such as a magnetic bead. Examples of target-capture methodologies are described by Ranki et al., U.S. Pat. No. 4,486,539, and Stabinsky, U.S. Pat. No. 4,751,177. Further uses of probes have been described, for example, in U.S. Pat. Nos. 5,210,015; 5,487,972; 5,804,375; 5,994,076.

[0008] HCV has been classified as the sole member of a distinct genus called *Hepacivirus* in the family Flaviviridae HCV is an enveloped particle harboring a plus-strand RNA with a length of approximately 9600 nucleotides. The genome carries a single long open reading frame (ORF) encoding a polyprotein that is proteolytically cleaved into a set of distinct products. An approximately 340 nucleotide-long 5' non-translated region (NTR) functions as an internal ribosome entry site (IRES) for translation of the HCV ORF

(Tsukiyama-Kohara et al., *J. Virol.* 66: 1476-1483 (1992); Wang et al., *J. Virol.* 67: 3338-3344 (1993)). The 3'NTR was more recently studied (Kolykhalov et al., *J. Virol.* 70: 3363-3371 (1996); Tanaka et al., *Biochem. Biophys. Res. Comm.* 215: 744-749 (1995), *J. Virol.* 70: 3307-3312 (1996); Yamada et al., *Virology* 223: 255-261 (1996)). The HCV 3'nontranslated region has been found to include four elements (positive sense, 5' to 3'): (i) a short sequence with significant variability among genotypes, (ii) a homopolymeric poly(U) tract, (iii) a polypyrimidine stretch consisting of mainly U with interspersed C residues, (iv) a novel sequence of 98 bases (the X-region). This latter nucleotide sequence is not present in human genomic DNA and is highly conserved among HCV genotypes (Kolykhalov et al. (1996); Pavio and Lai, J. Biosci. 28(3): 287-304 (2003)). Most HCV infections persist, leading in about 50% of all cases to chronic hepatitis, which can develop into chronic active hepatitis, liver cirrhosis and hepatocellular carcinoma. Furthermore, HCV is distributed worldwide, with the number of infected individuals being estimated to be ~170 million. (Bartenschlager and Lohmann (2000). Thus, a definitive diagnostic test is needed to identify infected individuals. Furthermore, HCV genotyping in patients is essential for diagnostic and epidemiological studies, as well as in studies of the natural history and treatment of HCV.

[**0009**] Detection of HCV RNA is the diagnostic test utilized for acute and chronic HCV infection. However, it is often complicated by the low levels of HCV replication or small numbers of infected cells in hepatitis C patients (Shi and Lai (2000)). Most qualitative and quantitative diagnostic tests are RTPCR aiming at the 5'-UTR, the most conserved region of the HCV genome. However, the sensitivity of this method has been reported to be accompanied by problems of false priming, presumably in areas of RNA secondary structure (Shi and Lai (2000)). This has been partially addressed by the use of tagged primers or a thermostable reverse transcriptase (Lanford, Virology 202: 606-614 (1994)). Since the core and NS5B genes are relatively conserved and do not contain extensive secondary structures, they have also been employed in various detection methods.

[**0010**] The X region of the 3'-UTR is another highly conserved region in the HCV genome, but it is highly structured. The 3' HCV NTR is considered a difficult amplification target for at least two reasons: it forms highly stable secondary structures; and the NTR is very small. It has not been used in the detection of HCV RNA due, at least in part, to its lack of practical advantages over the well-established tests based on the 5'-UTR sequence (Shi and Lai (2000)).

[**0011**] Thus there is a clear need for primer and probe designs that provide more useful and reliable results in detection of the presence of HCV. Specifically, there is a need for primers and probes that are aimed at overcoming the complications of 3' NTR secondary structure, type specificity and palindrome sequences that could affect the hybridization process and providing improved means for the detection, capture, and/or amplification of HCV nucleic acids. The present invention provides oligonucleotides that address these needs to help solve such issues.

SUMMARY OF THE INVENTION

[**0012**] The present invention provides an isolated Hepatitis C virus-derived nucleic acid comprising a nucleotide sequence selected from the group consisting of specific nucleic acid sequences corresponding to a portion of the 3'NTR of HCV. Such oligonucleotides are useful in detecting the presence of HCV nucleic acids. Specifically, the present invention provides an isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24; SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO:43.

[**0013**] The present invention further provides an isolated nucleic acid comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence corresponding to a portion of the 3'NTR of HCV, wherein the tag oligonucleotide consists of about 18 to about 23 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid. More specifically, the present invention further provides an isolated nucleic acid comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO: 8, wherein the tag oligonucleotide consists of about 18 to about 23 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid.

[**0014**] The present invention additionally provides a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of specific nucleic acid sequences corresponding to a portion of the 3'NTR of HCV, and (b) detecting the presence of amplified product. Specifically, the present invention additionally provides a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24; SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO:43, and (b) detecting the presence of amplified product.

[**0015**] The instant invention further provides a method of quantifying the amount of HCV nucleic acid in a sample comprising (a) amplifying selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of specific nucleic acid sequences corresponding to a portion of the 3'NTR of HCV, and (b) quantifying the amount of amplified product.

[**0016**] The instant invention further specifically provides a method of quantifying the amount of HCV nucleic acid in a sample comprising (a) amplifying selected HCV nucleic acid to form an HCV amplification product, utilizing as a

primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24; SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO:43, and (b) quantifying the amount of amplified product.

[0017] Additionally provided is a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a target nucleic acid comprising a selected portion of the 3'NTR of HCV and (b) detecting the presence of amplified product utilizing a probe comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22; SEQ ID NO:19, SEQ ID NO: 24, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 44.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] **FIG. 1** provides a schematic of the 3' NTR of HCV (98 sequences), showing the general region of hybridization of P1 primers, P2 primers and detection probes.

DETAILED DESCRIPTION OF THE INVENTION

[0019] The 3' HCV NTR is considered a difficult amplification target for at least two reasons. First, it forms highly stable secondary structures. The intramolecular pairing (including potential double-stranded (stem) and palindrome regions) is expected to cause difficulties for primer binding, primer extension, and probe binding. Secondly, the NTR is very small (98 nt), resulting in unusually limited primer and probe choices, and an amplicon of sub-optimal length (amplicons are ideally >100 nt). The inability to reduce secondary structure by raising the temperature (as in PCR) makes it particularly problematic for isothermal amplification methods such as TMA and NASBA. The present invention provides primers and probes that help overcome these challenges.

[0020] The 3' NTR of Hepatitis C virus has the following sequence:

Genotype 1:
GGUGGCUCCAUCUUAGCCCUAGUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCAUGACUG (SEQ ID NO: 25)

CAGAGAGUGCUGAUACUGGCCUCUCUGCAGAUCAUGU

Genotype 2:
GGUGGCUCCAUCUUAGCCCUAGUCACGGCUAGCUGUGAAAGGUCCGUGAGCCGCAUGACUG (SEQ ID NO: 26)

CAGAGAGUGCCGUAACUGGUCUCUCUGCAGAUCAUGU

[0021] The most common sequence differences between Genotype 1 and Genotype 2 are indicated by underlining. Although the region is highly conserved, additional nucleotide differences may also be present in the individual isolates.

[0022] As stated above, the present invention provides oligonucleotides, derived from the 3' NTR of Hepatitis C virus, found to be particularly useful in assays to detect the presence, or quantify the amount of, HCV nucleic acids in selected samples.

[0023] Throughout this application, nucleic acid sequences may have descriptors that include an "nt" range of numbers. Such descriptor indicates where, within the 98 nucleotides of the 3'HCV NTR, the sequence corresponds, the first of the 98 nucleotides, reading in a 5'-3' direction along the viral genome, having the number "1". For example, the following oligonucleotide "1206 (nt5-24)" indicates that the sequence of the oligonucleotide named 1206 has a nucleic acid sequence that corresponds to nucleotides 5-24 of the 3'NTR. Additionally, it is noted that references to sequences that include thymidine can be readily adapted to utilize uridine in substitution for thymidine, where useful for the particular assay. Furthermore, nucleotides may be modified by addition of chemical groups, or substitution of individual residues by analogues (e.g., 2'-O-methoxy versions). Additional such modified nucleotides are known in the art; some examples include hydroxymethyl nucleotides, methylated nucleotides, fluorinated nucleotides, alpha this phosphate nucleotides, amine-modified nucleotides, methoxy nucleotides, carboxymethyl nucleotides, thio nucleotides, inosine, dihydrouridine, psuedouridine, wybutosine, queuosine, C7dGTP. Additional modified nucleotides are found in U.S. Pat. Nos 5,405,950 and 5,633,364 (both, Mock and Lovern).

[0024] The invention particularly provides an isolated Hepatitis C virus-derived nucleic acid comprising a nucleotide sequence selected from the group consisting of specific nucleic acid sequences corresponding to a portion of the 3'NTR of HCV. Specifically, the present invention provides an isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO:32. It also provides an isolated nucleic acid consisting essentially of a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, and SEQ ID NO:32. Given the localization of useful target regions for hybridization of the primers and probes to the HCV 3' NTR, slight modifications, primarily mismatches from the provided sequences, are contemplated as being useful, provided they still hybridize to the target region of HCV under appropriate hybridization conditions. The nucleotide sequence of the oligonucleotide thus can further have from one to four mismatches from the recited nucleotide sequence, wherein the oligonucleotide hybridizes, under NASBA hybridization conditions, to the 3'NTR of HCV. NASBA conditions are known in the art and can include, for example, parameters creating stringency provided by about 41° C. and about 70 mM KCl (see. e.g., U.S.

Pat. Nos. 5,466,586; 5,409,818; 5,554,517; 6,063,603; and 5,130,238; Deiman B, van Aarle P, Sillekens P. Characteristics and applications of nucleic acid sequence-based amplification (NASBA).

[0025] Mol Biotechnol. 2002 February; 20(2):163-79.). Further, such sequences can have from one to four mismatches from HCV 3" NTR genomic sequence, wherein the oligonucleotide hybridizes, under NASBA hybridization conditions, to the 3'NTR of HCV. Examples of mismatches are exemplified herein.

[0026] Oligonucleotides of the present invention can be utilized, for example, as primers and/or as probes for the detection of HCV nucleic acids in a sample. Throughout this application, a particular oligonucleotide may be exemplified in use as a particular type (e.g. P1-type (linked to a sequence that provides a promoter region when in double-stranded form) or P2 type (used alone or linked to a tag oligonucleotide)) primer or as a probe; however, such use should not limit the use(s) for which the oligonucleotide may be useful. For example, a primer exemplified as a P1 primer may be useful as a P2-type primer. Additionally, an oligonucleotide exemplified for use as a probe may be useful as the base HCV-hybridizing sequence for other styles of probes (e.g., having different labels or capture oligonucleotides or other structures for functioning of that probe type).

[0027] The present invention provides amplicon length modulation through the use of the attachment of non-related sequence at the 5' end of primers. Such non-related sequences are particularly useful for non-promoter-linked primers, such as P2 primers herein. With this invention, our results demonstrate that one can, given the teachings herein, modulate amplicon length and modulate its secondary structure for highly efficient probe-based detection.

[0028] Thus, the present invention further provides an isolated nucleic acid comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence corresponding to a portion of the 3'NTR of HCV, wherein the tag oligonucleotide consists of about 18 to about 23 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid. More specifically, the present invention further provides an isolated nucleic acid comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO: 8, wherein the tag oligonucleotide consists of about 18 to about 23 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid.

[0029] In a preferred embodiment, the tag oligonucleotide (or "tail") is a sequence heterologous to the target nucleic acid, and, more preferably, heterologous to any region of HCV. The tag oligonucleotide can comprise any selected heterologous sequence of about 18-23 nucleotides. An approximately 20-mer oligonucleotide "tail" can readily be designed by one skilled in the art, given these teachings, to create additional detection tails useful with (non-promoter-carrying) primers to increase detectable amplicons from an amplification reaction. The tail is preferably comprised of sequences heterologous to the target nucleic acid. The oligonucleotide tail can be from about 18 to about 23 nucleotides in length, is preferably about 19-22 nucleotide in length, more preferably about 20-21 nucleotides in length, and most preferably 20 nucleotides in length. One example of an oligonucleotide tail is the "ECL (electrochemiluminescence) tail," set forth in SEQ ID NO:9.

[0030] In a specifically preferred embodiment, the tag oligonucleotide comprises an ECL tag; more particularly, the nucleotide sequence set forth in SEQ ID NO: 9. In a preferred embodiment, the oligonucleotide comprises a tag oligonucleotide linked at its 3' end to the 5' end of a primer having a nucleic acid sequence set forth in SEQ ID NO: 8. Thus, the present invention provides a preferred isolated nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO: 37, which consists of an ECL tag linked at its 3' end to the 5' end of the primer (herein referred to as "1259") having a nucleotide sequence set forth in SEQ ID NO:8.

[0031] The present invention further provides an isolated nucleic acid comprising (a) a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence comprising a nucleotide sequence corresponding to a portion of the 3'NTR of HCV. More specifically, the present invention provides an isolated nucleic acid comprising (a) a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, wherein the promoter oligonucleotide is linked at its 3' end to the 5' end of the primer nucleic acid.

[0032] The nucleic acid sequence of the T7 promoter is well-known to persons skilled in the art, and though a particular sequence is exemplified herein, functional equivalents having slight variations may be designed. In a preferred embodiment, the sequence of the T7 promoter is that set forth in SEQ ID NO:38. A further example is provided by SEQ ID NO: 23. An oligonucleotide having T7 promoter sequences is useful as a primer, and, when utilized as such, is herein referred to as a "P1 primer," P1-type primer, ""promoter-oligonucleotide," or simply as "P1." In a transcription-based amplification reaction (e.g., NASBA, TMA), as is known in the art, these T7 promoter sequences have a function in the amplification reaction, priming the transcription of RNA from the target template, in this case, HCV. The nucleotide sequences of primers exemplified herein as of the "P1" type are typically listed without the T7 promoter sequences, but in the experiments, such T7 promoter sequences are present.

[0033] Additionally provided is a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a target nucleic acid comprising a selected portion of the 3'NTR of HCV and (b) detecting the presence of amplified product utilizing a probe comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22; SEQ ID NO:19, SEQ ID NO: 24, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 44. In such a method, any useful HCV primer(s) can be utilized; examples are provided herein. Assay conditions are known to those of skill in the are and exemplified herein.

[0034] Oligonucleotides of the present invention can have various uses. They can, for example, be utilized as probes in capture and detection reactions, and as primers and/or probes in various amplification reactions. Provided herein are primers and probes utilized for 3' HCV amplification and exemplified by TMA-and NASBA. Both of these methods use isothermal amplification to produce RNA amplicons through the combined use of reverse transcriptase and T7 polymerase. Because of these similarities, the primers and probes are expected to share some performance similarity in the two systems, and experiments (see Examples) support this.

[0035] The present invention additionally provides a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of specific nucleic acid sequences corresponding to a portion of the 3'NTR of HCV, and (b) detecting the presence of amplified product. Specifically, invention provides a method of assaying for the presence of HCV in a nucleic acid sample comprising (a) amplifying a selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, and (b) detecting the presence of amplified product.

[0036] Nucleic acid hybridization techniques and conditions are known to the skilled artisan and have been described for example, in Sambrook et al. Molecular Cloning A Laboratory Manual, 2nd Ed. Cold Spring Lab. Press, December 1989; U.S. Pat. Nos. 4,563,419 (Ranki) and 4,851,330 (Kohne) and in Dunn, et al., *Cell* 12, pp. 23-26 (1978) among many other publications. Various modifications to the hybridization reactions are known in the art including in-solution hybridization or hybridization to capture probes on a solid support in one or more reaction steps.

[0037] Detection methods utilizing nucleic acids are also known. Nucleic acids can be labeled for use in detection. For example, detectable labels have been conjugated, directly or indirectly through linker arms on either the base, sugar or phosphate moiety of one or more specific oligonucleotides (see, e.g., U.S. Pat. Nos. 4,486,539 (Kourlisky); 4,411,955 (Ward); 4,882,269 (Schneider) and 4,213,893 (Carrico)). Labels known in the art include fluorochromes, radioisotopes, dyes, enzymes such as alkaline phosphatase, and luminescent or chemiluminescent molecules. Detectably labeled probes may, for example, be used to bind to amplified nucleic acid reaction products or amplicons during or after an amplification reaction. Particularly useful probes are those that can detect the target under the preferred conditions for the assay and type of probe utilized; typical hybridization conditions can be stringent hybridization conditions such as taught by Sambrook, et al.

[0038] This invention involves the primer and probe designs for efficient capture, amplification and detection of HCV 3' end 98-base non-translated region. The primer designs can be applied for TMA, NASBA, SDA, PCR and other amplification methods. The present oligonucleotides are useful in the present detection and quantification assays. Amplification can be performed by thermocycling methods or isothermal methods. The present oligonucleotides are particularly useful, and preferably used in, transcription based amplification methods, for example, NASBA and TMA. Transcription based amplification methods often utilize single stranded RNA as the input material, although single or double stranded DNA can likewise be used as input material. When a transcription based amplification method is practiced on a sample with single stranded RNA (of the "plus" sense) with additional sequences on both the 3'-end and the 5' end of the target sequence, a pair of oligonucleotides that is conveniently used with the methods can include (1) a first oligonucleotide (often referred to as a "promoter-oligonucleotide", or "P1" primer) that is capable of hybridizing to the 3-end of the target sequence, which oligonucleotide has the sequence of a promoter (preferably the T7 promoter) attached to its 5' end (the hybridizing part of this oligonucleotide has the opposite polarity as the plus RNA used as input material); and (2) a second oligonucleotide ("primer") which comprises the 3' end of the target sequence (this oligonucleotide has the same polarity as the plus RNA).

[0039] When such a pair of oligonucleotides, together with all enzymes having the appropriate activities, and a sufficient supply of the necessary ribonucleotides and deoxyribonucleotides are put together in one reaction mixture and are kept under the appropriate conditions (that is, under the appropriate buffer conditions and at the appropriate temperature) (such conditions being known in the art) for a sufficient period of time an isothermal continuous amplification reaction will start.

[0040] For use in non-transcription-based methods, the first primer can be an oligonucleotide provided here and can lack a T7 promoter sequence. For any amplification method, the present olgionucleotides can provide probes for detection of amplicons. The probe designs provided herein are for target capture (sample preparation), amplicon capture, and amplicon detection, which methods are know in the art. Probe designs for different detection methods: HPA, TAQman, molecular Beacons and Sandwich hybridization have also been discovered. These designs demonstrate the efficient capture and detection by hybridization to amplicons

EXAMPLES

[0041] Within this application and particularly within the Examples, the primers utilized may be referred to as P1 primers or P2 primers. This terminology simply indicates that, within a NASBA or TMA reaction in these examples, the P1 primer is the primer having a T7 promoter sequence attached to its 5' end (promoter primer); the P2 primer does not. It is not limiting terminology.

TMA Amplification and Detection of 3' HCV

[0042] To evaluate the usefulness of various primers in HCV amplification, standard VIDAS Probe D2 qHCV assay conditions were used as follows. For lysis, 0.5 ml sample (such as EDTA-plasma, or in vitro RNA transcript diluted in base matrix) was mixed with 0.4 ml urea-based lysis buffer, incubated 67.5° C. (20 min) and cooled to room temp (20 min). During these incubations the released nucleic acid was captured by capB probe (SEQ ID NO: 19), which was linked to magnetic beads by a polyA tail. Complexes were washed and resuspended in RAR buffer (see Table 1). RNA target

was then denatured at 65° C., then amplified by TMA (standard conditions; McDonough et al., *Nucleic acid amplification technologies* 1998:113-123 *BioTechniques Books* Natick, Mass., Lee H Morse S Olsvik Ø eds.) at 42° C. with primers 1259 (SEQ ID NO: 8) and 1236 (SEQ ID NO: 11). Specifically, the RAR-resuspended purified target was then added to VIDAS Probe strips (containing all additional reagents required for amplification and detection, such as enzymes, probes, wash solutions, and detection substrate). The amplification was carried out in bioMerieux AmpStations, and then transferred to VIDAS instruments, which carried out sequence-specific capture of the amplicons, washing, and detection with a fluorescent-conjugate probe specific for the 3' end. The SPR Probe 1247 (SEQ ID NO: 22) captures the amplicon on the VIDAS SPR. The AKP Probe (1246) (SEQ ID NO: 21) was conjugated to alkaline phosphatase and binds to the purified amplicon, providing detection via fluorescent AKP substrate.

TABLE 1

RAR Buffer	
Tween 80	12.5%
Tris pH 7.9	95 mM
EDTA	0.375 MM
KCl	50 MM
ZnAc	0.0765 MM
MgCl	20.75 MM
Glycerol	10%
DMSO	5%
ATP	4 MM
CTP	3 MM
GTP	6 MM
UTP	2 MM
d-ATPs	1 MM
d-CTPs	1 MM
d-GTPs	1 MM
d-TTPs	1 MM
Na Azide	0.06%
P1 Primer	40 NM

Additional Primer Sequences Used for TMA Amplification

[**0043**] Several P1 (T7 promoter) (SEQ ID NOs: 10-18) and P2 (reverse) (SEQ ID NOs: 1-8) primers (Table 3) were evaluated for 3' HCV amplification in the VIDAS Probe assay as described above. Among these, the 1236 (SEQ ID NO: 11)/1259 (SEQ ID NO: 8) primer pair was optimal, and derivatives of these (e.g., SEQ ID NO: 27 derived from 1236, SEQ ID NO: 37 derived from 1259) were also tested by NASBA (described below). Primer 1279 (SEQ ID NO: 15) also worked well for TMA and NASBA.

TABLE 2

Primer sequences		
P2 Primers		
1206	(SEQ ID NO: 1)	GCTCCATCTTAGCCCTAGTC
1242	(SEQ ID NO: 2)	TCTTAGCCCTAGTCA
1243	(SEQ ID NO: 3)	TTAGCCCTAGTCACG
1244	(SEQ ID NO: 4)	AGCCCTAGTCACGGC
1245	(SEQ ID NO: 5)	CCCTAGTCACGGCTA

TABLE 2-continued

Primer sequences		
1257	(SEQ ID NO: 6)	AGCCCTAGTCACGGCTAG
1258	(SEQ ID NO: 7)	AGCCCTAGTCACGGCTAGC
1259	(SEQ ID NO: 8)	CCCTAGTCACGGCTAGC
P1 primers		
1211	(SEQ ID NO: 10)	AGGCCAGTATCAGCACTCTC
1236	(SEQ ID NO: 11)	AGGCCAGTAACGGCACTCTCTGC
1237	(SEQ ID NO: 12)	AGGCCAGTATCGGCACTCTCTG
1238	(SEQ ID NO: 13)	AGGCCAGTAACGGCACTCUCTGC
1278	(SEQ ID NO: 14)	CAGTATCGGCACTCTCTGCAGT
1279	(SEQ ID NO: 15)	CCAGTATCGGCACTCTCTGCAG
1280	(SEQ ID NO: 16)	AGGCCAGTATCGGCACTCTCTGC
1281	(SEQ ID NO: 17)	AGTATCGGCACTCTCTGCAGT
1282	(SEQ ID NO: 18)	CAGTAACGGCACTCTCTGCAGT

NASBA Amplification of 3' HCV

[**0044**] The 1236/1259 (P1/P2) primer pair, which performed optimally in the TMA assay, was analyzed for use in NASBA amplification. Additional primers providing a larger predicted amplicon were also tested, for example primer P1: 1211 (nt83-64) (SEQ ID NO: 10) and primer P2:VP1206 (nt5-24) (SEQ ID NO: 1).

[**0045**] For detection of amplicons produced by NASBA, probes were designed to bind to a region corresponding to a combination of the VIDAS Probe amplicon capture probe and amplicon detection probe sites from the previous example. Probes were based upon an HCV base sequence of either TGAAAGGTCCGTGAGCCGC (nt 36-54) (SEQ ID NO: 43) or TGTGAAAGGTCCGTGAGCCGC (nt34-54) (SEQ ID NO: 44). These probes were tested as capture probes (EG6, EG7) in the ECL assay (see below) or as the amplicon-binding ("loop") portion of molecular beacons (MB140 (EG18) (SEQ ID NO: 24); MB102 (Q1) (SEQ ID NO: 41)

[**0046**] ECL (electrochemiluminescence) is a NASBA (bioMerieux, Inc., Durham, N.C.) endpoint detection system that detects amplicons after sequence-specific capture. The ECL system utilizes an ECL detection tag ("ECL tail") linked to the 5' end of a P2 primer. Utilizing an ECL detection tag (SEQ ID NO: 9) with above-described primers and capture probe EG7, amplification was considerable and better with the 1259/1236 pair than 1206/1211 (Table 3).

TABLE 3

Performance of various ECL-tagged P1 and P2 primers (normalized fluorescence values)

	HCV dilution					
P2, P1	1	10x	100x	1000x	10000x	neg
1259ECL, 1236	2.8E+07	3.3E+06	3.3E+04	9.9E+02	1.2E+02	1.0E+00
1206ECL, 1211	8.6E+02	2.9E+03	1.0E+00	1.0E+00	1.0E+00	1.0E+00

[0047] Similarly, in the absence of ECL tags the 1236 and 1259 primers performed better than 1206 and 1211 in NASBA reactions. As shown in table 4, the 1236/1259 pair performed well.

TABLE 4

Performance of various P1 and P2 primers (normalized fluorescence values).

P1 Primer:	1211	1211	1236	1236
P2 Primer:	1206	1259	1206	1259
Detection:	−0.0227978	0.0323256	0.0336202	0.7147294

ECL detection is presumed to be relatively insensitive to secondary structure, suggesting that the difference in sensitivity is more likely due to increased amplification than increased detection.

Modified P2 Primer Designs

[0048] In the above assays, it was also found that addition of the 20-nucleotide universal ECL tag to the P2 primer 1259 increased performance. Approximately a 10-fold increase in sensitivity was observed when using the ECL tail on the 1259 P2 primer, as compared to the P2 primer without the ECL tail (as shown in table 5, tagged detection of 10×-diluted sample is roughly equivalent to untagged detection of 1× sample).

TABLE 5

Comparison of ECL-tagged vs. untagged P2 primers (P1 used is 1236, probe is EG18, fluorescence values are normalized):

	HCV dilution:					
	1		10x		neg	
ECL tag	no	yes	no	yes	no	yes
Detection	0.80	3.58	0.13	0.97	0.09	0.09

[0049] ECL assays using an HCV-specific probe (i.e., not recognizing the ECL tag) also demonstrated improved performance with the tag, suggesting that this increase in performance was likely due to increased amplification (Table 6).

TABLE 6

Comparison of ECL-tagged 1259 vs. untagged, in ECL assay (P1 used is 1236, capture probe is EG7, fluorescence values are normalized):

	HCV dilution				
P2	1	10x	100x	1000x	neg
ECL-tagged 1259	3.2E+07	4.4E+06	2.0E+05	3.1E+03	1
1259	2.1E+06	6.9E+05	2.1E+04	1.3E+01	1

One likely explanation for the effect of the tag is that the HCV amplicon is very small (70 nt without tag, increased to 90 with tag), and small amplicons may be relatively inefficient in NASBA. Six P2 primers were designed to test whether additional extensions (of 10 or 20 additional nucleotides) to the ECL tag (either homologous to the target or heterologous) could improve amplification. None of the additional heterologous sequences increased performance, and additional homologous sequence decreased performance.

Additional P1 Primers for NASBA

[0050] Another set of P1 (promoter) primers was tested for function in NASBA, as listed in Table 7. This set of primers spans, and moves in the 3' direction in one-nucleotide increments, from the position of primer 1236 to (at the 3' end of the final primer P1.89) the position 3 bases from the probe-binding site. Of this set of primers, the best functioning (comparably to primer 1236) to produce detectable signal were P1.63, P1.82, P1.83, P1.84, and P1.85. The P2 used for these tests was EG4 and probe was EG18 (fluorescence values were normalized).

TABLE 7

P1.63	(SEQ ID NO: 27)	AGACCAGTTACGGCACTCTCTGC
P1.81	(SEQ ID NO: 28)	GACCAGTTACGGCACTCTCTGCA
P1.82	(SEQ ID NO: 29)	ACCAGTTACGGCACTCTGTGCAG
P1.83	(SEQ ID NO: 30)	CCAGTTACGGCACTCTCTGCAGT
P1.84	(SEQ ID NO: 31)	CAGTTACGGCACTCTCTGCAGTC
P1.85	(SEQ ID NO: 32)	AGTTACGGCACTCTCTGCAGTCA
P1.86	(SEQ ID NO: 33)	GTTACGGCACTCTCTGCAGTCAT
P1.87	(SEQ ID NO: 34)	TTACGGCACTCTCTGCAGTCATG
P1.88	(SEQ ID NO: 35)	TACGGCACTCTCTGCAGTCATGC
P1.89	(SEQ ID NO: 36)	ACGGCACTCTCTGCAGTCATGCG

[0051]

TABLE 8

Amplification with various P1 primers

Primer:	P1.63	P1.81	P1.82	P1.83	P1.84	P1.85	P1.86	P1.87	P1.88	P1.89
Signal:	5.250	3.184	4.886	5.099	5.239	3.746	2.668	1.995	0.025	0.142

[0052] Throughout this application, various publications are referenced. The disclosures of each of these publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[0053] Thus, while there have been described what are presently believed to be the preferred embodiments of the present invention, those skilled in the art will realize that other and further embodiments can be made without departing from the spirit and scope of the invention, and it is intended to include all such further modifications and changes as come within the true scope of the invention.

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<221> NAME/KEY: misc_feature
<222> LOCATION: (21)..(37)
<223> OTHER INFORMATION: "1259" portion

<400> SEQUENCE: 37

gatgcaaggt cgcatatgag ccctagtcac ggctagc 37

<210> SEQ ID NO 38
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Bacteriophage T7
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<223> OTHER INFORMATION: T7 promoter

<400> SEQUENCE: 38

aattctaata cgactcacta taggg 25

<210> SEQ ID NO 39
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<213> ORGANISM: Artificial
<220> FEATURE:
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<223> OTHER INFORMATION: "MB110 (Q2)"
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<223> OTHER INFORMATION: loop portion

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<400> SEQUENCE: 39

gcactcgtgt gaaaggtccg tgagccgccg agtgc 35

<210> SEQ ID NO 40
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<213> ORGANISM: Artificial
<220> FEATURE:
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<223> OTHER INFORMATION: “MB114”
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<222> LOCATION: (8)..(28)
<223> OTHER INFORMATION: loop portion

<400> SEQUENCE: 40

cgactcgtgt gaaaggtccg tgagccgccg agtcg 35

<210> SEQ ID NO 41
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<220> FEATURE:
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<223> OTHER INFORMATION: “MB102 (Q1)”
<220> FEATURE:
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<223> OTHER INFORMATION: loop portion

<400> SEQUENCE: 41

gcatgctgtg aaaggtccgt gagccgcgca tgc 33

<210> SEQ ID NO 42
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Hepatitis C virus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(21)
<223> OTHER INFORMATION: “EG6”

<400> SEQUENCE: 42

tgtgaaaggt ccgtgagccg c 21

<210> SEQ ID NO 43
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Hepatitis C virus
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(19)
<223> OTHER INFORMATION: “EG7”

<400> SEQUENCE: 43

tgaaaggtcc gtgagccgc 19

1. An isolated nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO:24, SEQ ID NO:41, SEQ ID NO:43, and SEQ ID NO:42.

2. An isolated nucleic acid consisting essentially of a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO:24, SEQ ID NO:41, SEQ ID NO:43, and SEQ ID NO:42.

3. An isolated nucleic acid consisting essentially of a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO:24, SEQ ID NO:41, SEQ ID NO:43, and SEQ ID NO:42, wherein said nucleotide sequence further has from one to four mismatches and hybridizes under NASBA hybridization conditions, to the 3'NTR of HCV.

4. An isolated nucleic acid comprising a nucleotide sequence set forth in SEQ ID NO: 37.

5. An isolated nucleic acid consisting essentially of a nucleotide sequence set forth in SEQ ID NO:37.

6. An isolated nucleic acid comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO: 8, wherein the tag oligonucleotide consists of about 18 to about 23 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid.

7. The isolated nucleic acid of claim 6, wherein the tag oligonucleotide comprises the nucleotide sequence set forth in SEQ ID NO: 9.

8. An isolated nucleic acid comprising (a) a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO: 15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, wherein the promoter oligonucleotide is linked at its 3' end to the 5' end of the primer nucleic acid.

9. The isolated nucleic acid of claim 8, wherein the promoter oligonucleotide has the nucleic acid sequence set forth in SEQ ID NO:38.

10. The isolated nucleic acid of claim 8, wherein the promoter oligonucleotide has the nucleic acid sequence set forth in SEQ ID NO:23.

11. A method of assaying for the presence of HCV in a nucleic acid sample comprising

a. Amplifying a selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24; SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO:43; and

b. Detecting the presence of amplified product.

12. The method of claim 11, wherein the amplification utilizes a first primer comprising the nucleotide sequence set forth in SEQ ID NO:11 and a second primer comprising the nucleotide sequence set forth in SEQ ID NO:8.

13. The method of claim 12, wherein the first primer further comprises, at its 5' end, a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter.

14. The method of claim 13, wherein the T7 promoter is SEQ ID NO: 38.

15. The method of claim 11, wherein the amplification utilizes a first primer comprising the nucleotide sequence set forth in SEQ ID NO: 15 and a second primer comprising the nucleotide sequence set forth in SEQ ID NO:8.

16. The method of claim 15, wherein the first primer further comprises, at its 5' end, a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter.

17. The method of claim 11, wherein the amplification utilizes a first primer and a second primer, said second primer comprising (a) a tag oligonucleotide having a 5' end and a 3' end, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and SEQ ID NO: 8, wherein the tag oligonucleotide consists of about 10 to about 25 randomly selected nucleotides heterologous to HCV and is linked at its 3' end to the 5' end of the primer nucleic acid.

18. The method of claim 11, wherein the amplification utilizes a first primer and a second primer, said first primer comprising (a) a promoter oligonucleotide that, when in double-stranded form, can function as a T7 promoter, and (b) a primer nucleic acid having a 5' end and a 3' end and comprising a nucleotide sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32, wherein the promoter oligonucleotide is linked at its 3' end to the 5' end of the primer nucleic acid.

19. The method of claim 11, wherein the detecting is performed by hybridizing to the amplification product a detectable probe.

20. The method of claim 19, wherein the detectable probe comprises a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43.

21. The method of claim 11, wherein the detecting is performed by capturing the amplification product on an immobilised capture probe.

22. The method of claim 21, wherein the capture probe comprises a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 19.

23. The method of claim 11, wherein the amplification is performed by isothermal amplification or thermocyclic amplification.

24. A method of assaying for the presence of HCV in a nucleic acid sample comprising

a. Amplifying a target nucleic acid comprising a selected portion of the 3'NTR of HCV and

b. Detecting the presence of amplified product utilizing a probe comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 21, SEQ ID NO: 22; SEQ ID NO:19, SEQ ID NO: 24, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO: 43.

25. A method of detecting the presence of HCV nucleic acid in a sample comprising hybridizing with the sample, under selective hybridization conditions, a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32.

26. A method of quantifying the amount of HCV nucleic acid in a sample comprising

a. Amplifying selected HCV nucleic acid to form an HCV amplification product, utilizing as a primer of amplification a nucleic acid comprising a nucleotide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 11, SEQ ID NO: 15, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 24; SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 30, SEQ ID NO: 31, SEQ ID NO:32, SEQ ID NO: 41, SEQ ID NO: 42, and SEQ ID NO:43, and

b. Quantifying the amount of amplified product.

27. The method of claim 26, wherein the amplification utilizes a first primer comprising the nucleotide sequence selected from the group consisting of SEQ ID NO:11 and SEQ ID NO: 15 and a second primer comprising the nucleotide sequence set forth in SEQ ID NO:8.

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