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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING GENE EXPRESSION OF HEPATITIS B VIRUS

(57) Abrégé/Abstract:
The invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a Hepatitis B Virus gene. The invention also relates to a pharmaceutical composition comprising the dsRNA or nucleic acid molecules or vectors encoding the same together with a pharmaceutically acceptable carrier; methods for treating diseases caused by Hepatitis B Virus infection using said pharmaceutical composition: and methods for inhibiting the expression of a Hepatitis B Virus gene in a cell.

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(54) **Title**: COMPOSITIONS AND METHODS FOR INHIBITING GENE EXPRESSION OF HEPATITIS B VIRUS

(57) **Abstract**: The invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a Hepatitis B Virus gene. The invention also relates to a pharmaceutical composition comprising the dsRNA or nucleic acid molecules or vectors encoding the same together with a pharmaceutically acceptable carrier; methods for treating diseases caused by Hepatitis B Virus infection using said pharmaceutical composition: and methods for inhibiting the expression of a Hepatitis B Virus gene in a cell.

Compositions and Methods for Inhibiting Gene Expression of Hepatitis B Virus

BACKGROUND OF THE INVENTION

This invention relates to double-stranded ribonucleic acids (dsRNAs), and their use in mediating RNA interference to inhibit the expression of genes, necessary for replication and pathogenesis of Hepatitis B Virus, in particular in the inhibition of viral polymerase, surface antigen, e-antigen and the X protein. Furthermore, the use of said dsRNAs to treat or prevent chronic liver diseases/disorders, inflammations, fibrotic conditions and proliferative disorders, like cancers, as consequence of Hepatitis B Virus infection, is part of this invention.

The Hepatitis B Virus is a strict hepatotrophic, double-stranded DNA containing virus. Although DNA is the genetic material, the replication cycle involves a reverse transcription step to copy a pregenomic RNA into DNA. In order to accomplish this essential step, the viral-encoded polymerase possesses reverse transcriptase activity. Hepatitis B virus is classified as one member of the Hepadnaviruses and belongs to the family of Hepadnaviridae. The primary infection of adult humans with Hepatitis B Virus causes an acute hepatitis with symptoms of organ inflammation, fever, jaundice and increased liver transaminases in blood. About 95% of acute hepatitis resolve without treatment. Those patients, that are not able to overcome the virus infection, suffer a chronic disease progression over many years with increased risk of developing cirrhotic liver or liver cancer. Perinatal transmission from Hepatitis B virus-infected mothers to newborns also leads to chronic hepatitis. The treatment options for chronic Hepatitis B Virus infection are limited and lead only in some cases to complete and lasting remission. Additional clinical and therapeutical complications arise in Hepatitis B Virus patients co-infected with Hepatitis C, Hepatitis D or Human Immunodeficiency Virus.

The Hepatitis B Virus is transmitted via blood or blood products, sperm, vaginal secrets, or saliva. Drug abuse and sexual intercourse are dangerous activities and support spreading the virus. Contact of damaged, mucoid epithelia with contaminated body fluids may be sufficient for infection. Incubation time is between 40 to 200 days. The risk for infection is proportional to the number of transmitted Hepatitis B Virus particles. Babies are often infected perinatally by their Hepatitis B Virus carrying mother, a major health problem in endemic areas.

About 2 billion people are infected with Hepatitis B Virus and 400 million are chronic carriers. Areas with high prevalence are Africa and South-East Asia, with local accumulation of 20-80% infected persons.

Based on sequence homology, Hepatitis B Viruses are classified into genotypes A-H, with genotypes A-D being the most important ones. Genotype A is frequent in North-Western Europe, USA, South and Central America. Genotype B and C are dominant in China, Japan, Indonesia and other countries in East Asia. Genotype D is found in Southern Europe, Northern Africa and South Africa. Disease progression and response to pharmaceutical treatment differ among genotypes.

Infectious Hepatitis B Virus particles have a diameter of about 42 nm. The outer membrane bilayer contains the large, middle and small surface protein. The cognate hepatocellular receptor for virus surface protein binding and internalization is unknown. Many copies of core protein form a spherical nucleocapsid structure inside the virus particle. Each nucleocapsid carries partial double-stranded DNA as genetic material, together with viral polymerase.

Upon uptake by hepatocytes, the nucleocapsid is transferred to the nucleus and DNA is released. There, the DNA strand synthesis is completed and gaps repaired to give the covalently closed circular (ccc) supercoiled DNA of 3.2kb. The cccDNA serves as template for transcription of four major viral mRNAs, which are 3.5, 2.4, 2.1 and 0.7 kb long. All mRNAs are 5′-capped and polyadenylated at the 3′-end. There is sequence overlap at the 3′-end between all four mRNAs.

The 3.5 kb mRNA serves as template for core protein and polymerase production. In addition, the same transcript serves as a pre-genomic replication intermediate and allows the viral polymerase to initiate the reverse transcription into DNA. Core protein is needed for nucleocapsid formation. In addition, sequential processing activities transforms some core protein into the secretable e-antigen. The abundance of e-antigen in blood correlates with Hepatitis B Virus replication in liver and serves as important diagnostic marker for monitoring the disease progression.

The 2.4 and 2.1 kb mRNAs carry the open reading frames pre-S1, pre-S2 and S2 for expression of viral large, medium and small surface antigen. The s-antigen is associated with infectious, complete particles. In addition, blood of infected patients also contain non-infectious particles derived from s-antigen alone, free of genomic DNA or polymerase. The function of these particles is not fully understood. The complete and lasting depletion of detectable s-antigen in blood is considered as reliable indicator for Hepatitis B Virus clearance and thus, successful cure.

The 0.7 kb mRNA encodes the X protein. This gene product is important for efficient transcription of viral genes and also acts as a transactivator on host gene expression. The latter activity seems to be important for hepatocyte transformation during development of liver cancer.

Recombinant Hepatitis B Virus s-antigen is used for vaccination. The injection of three doses of formulated s-antigen at day 1, at 4 weeks and at 6 months usually induces a sufficient titer of neutralizing antibodies. Vaccinated patients are protected for 10 years or longer. However, the vaccines are no substitute for therapy.

Patients with acute Hepatitis B Virus infection are not treated due to the high, natural remission rate. However, those patients with detectable s-antigen, e-antigen or viral DNA in the blood for more than 6 months are considered chronically infected. Nucleoside analogs as inhibitors of reverse transcriptase activity are the first treatment option for many patients. Lamivudine, Tenofovir, or Entecavir suppress Hepatitis B Virus replication, sometimes to undetectable levels. Improvement of liver function and reduction of liver inflammation are the most important benefits. However, only few patients achieve complete and lasting remission after the end of treatment. Furthermore, the Hepatitis B Virus develops drug resistance with increasing duration of treatment. This is especially difficult for patients co-infected with Hepatitis B and Human Immunodeficiency Virus. Both viruses are susceptible to nucleoside analogue drugs and may co-develop resistance.

The second treatment option is the administration of interferon-alpha. Here, patients receive high doses of interferon-alpha over a period of 6 months. Depending on the virus genotype, up to 50% of chronic infection are curable. However, the Asian genotype B gives very poor response rates. Co-infection with Hepatitis D or Human

Immunodeficiency Virus renders interferon-alpha therapy completely ineffective. Patients with strong liver damage and heavy fibrotic conditions are not qualified for interferon-alpha therapy.

Despite significant advances in the field of Hepatitis B Virus treatment, there remains a need for an agent that can selectively and efficiently silence the gene expression of the virus, blocks replication and subsequently reduces viral burden in chronically infected patients.

Double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). The invention provides double-stranded ribonucleic acid molecules (dsRNAs), as well as compositions and methods for inhibiting the expression of the Hepatitis B Virus gene, in particular the expression of the Hepatitis B Virus gene, in a cell, tissue or mammal using such dsRNA. The invention also provides compositions and methods for treating or preventing pathological conditions and diseases caused by the infection of the Hepatitis B Virus such as in chronic liver diseases/disorders, inflammations, fibrotic conditions and proliferative disorders, like cancers.

SUMMARY OF THE INVENTION

The invention provides double-stranded ribonucleic acid (dsRNA) molecules able to selectively and efficiently decrease the expression of Hepatitis B Virus gene. The use of Hepatitis B Virus RNAi provides a method for the therapeutic and/or prophylactic treatment of diseases/disorders which are associated with chronic liver diseases/disorders, inflammations, fibrotic conditions and proliferative disorders, like cancers, such method comprises administration of dsRNA targeting Hepatitis B Virus to a human being or animal.

In one preferred embodiment the described dsRNA molecule is capable of inhibiting the expression of a Hepatitis B Virus gene by at least 60%, preferably by at least 70%, most preferably by at least 80%.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of a Hepatitis B Virus gene, in particular the

expression of the genes related to replication or pathogenesis of Hepatitis B Virus. The dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence, see sequences provided in the sequence listing and also the specific dsRNA pairs in the appended Table 1 and Table 2. In one embodiment the sense strand comprises a sequence which has an identity of at least 90% to at least a portion of an Hepatitis B Virus mRNA . Said sequence is located in a region of complementarity of the sense strand to the antisense strand, preferably within nucleotides 2-7 of the 5' terminus of the antisense strand. In one preferred embodiment the dsRNA specifically targets the Hepatitis B Virus gene that encodes core protein, viral polymerase, surface antigen, e-antigen or the X protein. Furthermore, it is preferred that the dsRNA specifically targets a consensus sequence which has a highly conserved nucleic acid sequence among the Hepatitis B Virus genomic sequences of genotype A, B, C and D. Preferably, the consensus sequence is at least 13 contiguous nucleotides in length, more preferably at least 17 contiguous nucleotides, and most preferably at least 19 contiguous nucleotides. Preferred highly conserved nucleic acid sequences are listed in Table 5.

In one embodiment, the antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding said Hepatitis B Virus gene, and the region of complementarity is most preferably less than 30 nucleotides in length. Furthermore, it is preferred that the length of the herein described inventive dsRNA molecules (duplex length) is in the range of about 16 to 30 nucleotides, in particular in the range of about 18 to 28 nucleotides. Particularly useful in context of this invention are duplex lengths of about 19, 20, 21, 22, 23 or 24 nucleotides. Most preferred are duplex stretches of 19, 21 or 23 nucleotides. The dsRNA, upon delivery to a cell infected by a Hepatitis B Virus, inhibits the expression of a Hepatitis B Virus gene *in vitro* by at least 60%, preferably by at least 70%, and most preferably by 80%.

The invention further provides a double-stranded ribonucleic acid molecule capable of inhibiting the expression of a Hepatitis B Virus gene *in vitro* by at least 80% wherein said double-stranded ribonucleic acid molecule comprises a sense strand comprising in order nucleotides 1-19 of SEQ ID 2, 4, 6, or 7 and an antisense strand at least partially complementary to the sense strand and wherein said sequence is less than 30 nucleotides in length.

The invention further provides a double-stranded ribonucleic acid molecule capable of inhibiting the expression of a Hepatitis B Virus gene wherein said double-stranded ribonucleic acid molecule comprises a sense strand comprising in order nucleotides 1-19 of SEQ ID NO: 2, 3, or 6 and an antisense strand at least partially complementary to the sense strand and wherein said sense strand is less than 30 nucleotides in length. In an embodiment, the sense strand comprises in order nucleotides 1-19 of SEQ ID NO: 2.

The invention further provides a double-stranded ribonucleic acid molecule capable of inhibiting the expression of a Hepatitis B Virus gene *in vitro* by at least 80% wherein said double-stranded ribonucleic acid molecule comprises a sense strand comprising in order nucleotides 1-19 of SEQ ID NO: 2, 3, or 6 and an antisense strand at least partially complementary to the sense strand and wherein said sequence is less than 30 nucleotides in length.

The invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier, stabilizer and/or diluent and:

a) at least one double-stranded ribonucleic acid molecule as defined herein;

b) at least one nucleic acid sequence encoding a sense strand or an antisense strand comprising the double-stranded ribonucleic acid molecule as defined herein; or,

c) a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined herein.

The invention further provides a pharmaceutical composition comprising a pharmaceutically acceptable carrier, stabilizer and/or diluent and:

a) first and second double-stranded ribonucleic acid molecules each as defined herein;

b) at least one nucleic acid sequence encoding sense strands or antisense strands comprising first and second double-stranded ribonucleic acid molecules each as defined herein; or,

c) a cell, tissue or non-human organism comprising first and second double-stranded ribonucleic acid molecules each as defined herein.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1. Table 1. Core sequences of dsRNAs targeting Hepatitis B Virus gene. Letters in capitals represent RNA nucleotides.

FIG. 2 Table 2. Characterization of dsRNAs targeting Hepatitis B Virus : Activity testing with single dose. Letters in capitals represent RNA nucleotides, lower case letters "c", "g", "a" and "u" represent 2′ O-methyl-modified nucleotides, "s"

represents phosphorothioate, "dT" represents deoxythymidine, upper case letters A, C, G, U followed by "f" indicates a 2′-fluoro nucleotide. Lower case "p" indicates a 5′-phosphate. (invdT) represents an inverted deoxythimidine (3′-3′-linked).

FIG. 3. Table 3. Characterization of dsRNAs targeting Hepatitis B Virus: Stability. t½ = half-life of a strand as defined in examples.

FIG. 4. Table 4. Core sequences of dsRNAs targeting Hepatitis B Virus gene and their modified counterparts. Letters in capitals represent RNA nucleotides, lower case letters "c", "g", "a" and "u" represent 2′ O-methyl-modified nucleotides, "s" represents phosphorothioate, "dT" represents deoxythymidine, upper case letters A, C, G, U followed by "f" indicates a 2′-fluoro nucleotide. Lower case "p" indicates a 5′-phosphate. (invdT) represents an inverted deoxythimidine (3′-3′-linked).

FIG. 5. Table 5. Target site sequences of dsRNAs targeting Hepatitis B Virus and their coverage rate with respect to Hepatitis B Virus genotypes A, B, C and D. n = number of available HBV sequences of each genotype

FIG. 6. Table 6. NCBI Genbank accession Nos. of Hepatitis B Virus genomic sequences.

FIG. 7. Table 7. Comparision of knockdown efficacies and coverage of HBV genomes for single dsRNAs and combinations thereof. Activity testing for combinations of two dsRNAs was done at final concentrations of 10 nM and at 1 nM with the best performing dsRNAs according to Table 2 and compared with respective data.

FIG. 8. Table 8. Sequences of the negative control ds RNAs used in the psiCHECK™-2 screening assay.

DETAILED DESCRIPTION OF THE INVENTION

Appended Table 1 relates to preferred molecules to be used as dsRNA in accordance with this invention. Also modified dsRNA molecules are provided herein and are in particular disclosed in appended Table 2, providing illustrative examples of modified dsRNA molecules of the present invention. As pointed out herein above, Table 2 provides for illustrative examples of modified dsRNAs of this invention (whereby the corresponding sense strand and antisense strand is provided in this Table). The relation of the unmodified preferred molecules shown in Table 1 to the modified dsRNAs of Table 2 is illustrated in Table 4. Yet, the illustrative modifications of these constituents of the inventive dsRNAs

are provided herein as examples of modifications.

Table 3 provides for selective biological, clinical and pharmaceutical relevant parameters of certain dsRNA molecules of this invention.

Some of the preferred dsRNA molecules are provided in the appended Table 1 and, inter alia and preferably, wherein the sense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 1, 2, 3, 4, 5, 6, 7, and 26. The antisense strand is selected from the group consisting of the nucleic acid sequences depicted in SEQ ID NOs: 157, 158, 160, 161, 162, 163, 164, and 186. Accordingly, the inventive dsRNA molecule may, inter alia, comprise the sequence pairs selected from the group consisting of SEQ ID NOs: 1/157, 2/158, 3/160, 4/161, 5/162, 6/163, 7/164, and 26/186. In the context of specific dsRNA molecules provided herein, pairs of SEQ ID NOs relate to corresponding sense and antisense strands sequences (5′ to 3′) as also shown in the Tables.

In one embodiment the dsRNA molecules comprise an antisense strand with a 3′ overhang of 1-5 nucleotides in length, preferably 1-2 nucleotides in length. Preferably said overhang of the antisense strand comprises uracil or nucleotides which are complementary to the mRNA encoding a protein necessary for replication or pathogenesis of Hepatitis B Virus, in particular core protein, viral polymerase, surface antigen, e-antigen and X protein. In another preferred embodiment, said dsRNA molecules comprise a sense strand with a 3′ overhang of 1-5 nucleotides in length, preferably 1-2 nucleotides in length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are identical to the mRNA encoding a protein necessary for replication or pathogenesis of Hepatitis B Virus .

In another preferred embodiment, the dsRNA molecules comprise a sense strand with a 3′ overhang of 1-5 nucleotides in length, preferably 1-2 nucleotides in length, and an antisense strand with a 3′ overhang of 1-5 nucleotides in length, preferably 1-2 nucleotides in length. Preferably said overhang of the sense strand comprises uracil or nucleotides which are at least 90% identical to the pregenomic RNA and/or the mRNA encoding the protein necessary for replication or pathogenesis of Hepatitis B Virus and said overhang of the antisense strand comprises uracil or nucleotides which are at least 90% complementary to the mRNA encoding the protein necessary for replication or pathogenesis of Hepatitis B Virus .

The dsRNA molecules of the invention may be comprised of naturally occurring nucleotides or may be comprised of at least one modified nucleotide, such as a 2′-O-methyl modified nucleotide, inverted deoxythymidine, a nucleotide comprising a 5′-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group. 2′ modified nucleotides may have the additional advantage that certain immunostimulatory factors or cytokines are suppressed when the inventive dsRNA molecules are employed *in vivo*, for example in a medical setting. Alternatively and non-limiting, the modified nucleotide may be chosen from the group of: a 2′-deoxy-2′-fluoro modified nucleotide, a 2′-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2′-amino-modified nucleotide, 2′-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. In one preferred embodiment the dsRNA molecules comprises at least one of the following modified nucleotides: a 2′-O-methyl modified nucleotide, a nucleotide comprising a 5′-phosphorothioate group and a deoxythymidine. Preferred dsRNA molecules comprising modified nucleotides are given in Table 2. In another preferred embodiment one of those deoxythymidine nucleotides at the 3′ of both strand is an inverted deoxythymidine.

In a preferred embodiment the inventive dsRNA molecules comprise modified nucleotides as detailed in the sequences given in Table 2. In one preferred embodiment the inventive dsRNA molecule comprises sequence pairs selected from the group consisting of SEQ ID NOs: 1/157, 2/158, 3/160, 4/161, 5/162, 6/163, 7/164, and 26/186, and comprises overhangs at the antisense and/or sense strand of 1-2 deoxythymidines. In one preferred embodiment the inventive dsRNA molecule comprises sequence pairs selected from the group consisting of SEQ ID NOs: 1/157, 2/158, 3/160, 4/161, 5/162, 6/163, 7/164, and 26/186, and comprise modifications as detailed in Table 2. Preferred dsRNA molecules comprising modified nucleotides are listed in Table 2-4, with the most preferred dsRNA molecules depicted in SEQ ID Nos: 321/485, 322/486, 324/488, 325/489, 326/490, 327/491, 328/492, and 350/514.

In another embodiment, the inventive dsRNAs comprise modified nucleotides on positions different from those disclosed in Table 2. In one preferred embodiment two deoxythymidine nucleotides are found at the 3′ of both strands of the dsRNA molecule.

Preferably said deoxythymidine nucleotides form an overhang.

In one embodiment the dsRNA molecules of the invention comprise a sense and an antisense strand wherein both strands have a half-life of at least 0.9 h. In one preferred embodiment the dsRNA molecules of the invention comprise a sense and an antisense strand wherein both strands have a half-life of at least 48 h, preferably in human serum.

In another embodiment, a nucleic acid sequence encoding a sense strand and/or an antisense strand comprised in the dsRNAs as defined herein are provided.

The invention also provides for cells comprising at least one of the dsRNAs of the invention. The cell is preferably a mammalian cell, such as a human cell. Furthermore, tissues and/or non-human organisms comprising the herein defined dsRNA molecules are an embodiment of this invention, whereby said non-human organisms are particularly useful for research purposes or as research tools, for example in drug testing.

Furthermore, the invention relates to a method for inhibiting the expression of a Hepatitis B Virus gene, in particular a Hepatitis B Virus gene that encodes core protein, viral polymerase, surface antigen, e-antigen or the X protein, in a cell, tissue or organism comprising the following steps:

(a) introducing into the cell, tissue or organism a double-stranded ribonucleic acid (dsRNA) as defined herein; and

(b) maintaining said cell, tissue or organism produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of a Hepatitis B Virus gene, thereby inhibiting expression of a Hepatitis B Virus gene in a given cell.

The invention also relates to pharmaceutical compositions comprising at least one kind of the inventive dsRNAs. These pharmaceutical compositions are particularly useful in the inhibition of the expression of a Hepatitis B Virus gene in a cell, a tissue or an organism.

Preferably said at least one kind of the inventive double-stranded ribonucleic acid molecules target the region of a pregenomic RNA and/or a mRNA encoding a protein necessary for replication or pathogenesis of Hepatitis B Virus gene. More preferably said target region of the inventive double-stranded ribonucleic acid molecules comprises a

consensus sequence which is highly conserved among the Hepatitis B Virus genomic sequences of genotype A, B, C and D, and said consensus sequence is at least 13 contiguous nucleotides in length, preferably at least 17 contiguous nucleotides, most preferably at least 19 contiguous nucleotides. Preferred highly conserved nucleic acid sequences are listed in Table 5. The pharmaceutical compositions may be used to treat patients who are infected with any genotype of Hepatitis B Virus or co-infected with different genotypes of Hepatitis B Virus.

In case the pharmaceutical composition comprises at least two kinds of inventive double-stranded ribonucleic acid molecules, it is preferable that the targets of said double-stranded ribonucleic acid molecules are different from each other. The inventive pharmaceutical compositions may be used to treat the patients and to prevent the Hepatitis B Virus from developing resistance to the pharmaceutical compositions. In a preferred embodiment the inventive pharmaceutical compositions comprise the combination of dsRNA molecules as detailed in the sequences given in Table 7. In one preferred embodiment the inventive pharmaceutical compositions comprise combinations of dsRNA pairs selected from the group consisting of SEQ ID NOs: 322/486 and 333/497, 322/486 and 346/510, 322/486 and 330/494, and, 322/486 and 324/488.

The pharmaceutical compositions described above may also comprise (a) pharmaceutically acceptable carrier(s), diluent(s) and/or excipient(s).

In another embodiment, the invention provides methods for treating, preventing or managing chronic liver diseases/disorders, inflammations, fibrotic conditions and/or proliferative disorders like cancers which are associated with Hepatitis B Virus, said method comprising administering to a subject in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention. Preferably, said subject is a mammal, most preferably a human patient.

In one embodiment, the invention provides a method for treating a subject having a pathological condition mediated by the infection of a Hepatitis B Virus. Such conditions comprise disorders associated with chronic liver diseases/disorders, inflammations, fibrotic conditions and/or proliferative disorders like cancers, as described above. In this

embodiment, the dsRNA acts as a therapeutic agent for controlling the expression of a Hepatitis B Virus gene. The method comprises administering a pharmaceutical composition of the invention to the patient (e.g., human), such that expression of a Hepatitis B Virus gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target mRNAs of a Hepatitis B Virus gene. In one preferred embodiment the described dsRNAs specifically decrease Hepatitis B Virus mRNA levels and do not directly affect the expression and/or mRNA levels of off- target genes in the cell.

In one preferred embodiment the described dsRNA decrease Hepatitis B Virus mRNA levels in the liver by at least 60%, preferably by at least 70%, most preferably by at least 80% *in vivo*. In another embodiment the described dsRNAs decrease Hepatitis B Virus mRNA levels *in vivo* for at least 4 days. In another preferred embodiment, the dsRNAs of the invention are used for the preparation of a pharmaceutical composition for the treatment of chronic liver diseases/disorders, inflammations, fibrotic conditions and proliferative disorders like cancers. Such diseases to be treated with said pharmaceutical composition comprise but are not limited to: chronic hepatitis (CH), hepatic cirrhosis (HC) and hepatocellular carcinoma (HCC).

In another embodiment, the invention provides vectors for inhibiting the expression of a Hepatitis B Virus gene in a cell, in particular a Hepatitis B Virus gene comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of the dsRNA molecules of the invention.

In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of a Hepatitis B Virus gene in a cell. Said vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of the dsRNA molecule of the invention. Yet, it is preferred that said vector comprises, besides said regulatory sequence a sequence that encodes at least one "sense strand" of the inventive dsRNA and at least one "antisense strand" of said dsRNA. It is also envisaged that the claimed cell comprises two or more vectors comprising, besides said regulatory sequences, the herein defined sequence(s) that encode(s) at least one strand of the dsRNA molecules of the invention.

In one embodiment, the method comprises administering a composition comprising a dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of a Hepatitis B Virus gene of the mammal to be treated. As pointed out above, also vectors and cells comprising nucleic acid molecules that encode for at least one strand of the herein defined dsRNA molecules can be used as pharmaceutical compositions and may, therefore, also be employed in the herein disclosed methods of treating a subject in need of medical intervention. It is also of note that these embodiments relating to pharmaceutical compositions and to corresponding methods of treating a (human) subject also relate to approaches like gene therapy approaches.

Hepatitis B Virus specific dsRNA molecules as provided herein or nucleic acid molecules encoding individual strands of these inventive dsRNA molecules may also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. *Proc. Natl. Acad. Sci.* USA (1994) 91:3054-3057). The pharmaceutical preparation of the gene therapy vector can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

In another aspect of the invention, Hepatitis B Virus specific dsRNA molecules that modulate Hepatitis B Virus gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Skillern A et al., International PCT Publication No. WO 00/22113). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann et al., *Proc. Natl. Acad. Sci.* USA (1995) 92:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the

same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are preferably DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka et al., *Curr. Topics Micro. Immunol.* (1992) 158:97-129); adenovirus (see, for example, Berkner et al., *BioTechniques* (1998) 6:616; Rosenfeld et al. *Science* (1991) 252:431-434; and Rosenfeld et al. *Cell* (1992) 68:143-155); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, e.g., Danos and Mulligan, *Proc. NatI. Acad. Sci.* USA (1998) 85:6460-6464). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette et al., *Human Gene Therapy* (1991) 2:5-10; Cone et al., *Proc. Natl. Acad. Sci.* USA (1984) 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., *J. Infectious Disease*, (1992) 166:769), and also have the advantage of not requiring mitotically active cells for infection.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U1 snRNA promoter) or preferably RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., *Proc. Natl. Acad. Sci.* USA (1986) 83:2511-2515).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., *FASEB J.* (1994) 8:20-24). Such inducible expression systems,

suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Preferably, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single Hepatitis B Virus gene or multiple Hepatitis B Virus genes over a period of a week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of *ex vivo* cells can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of a target Hepatitis B Virus gene, as well as compositions and methods for treating diseases and disorders caused by the infection of said Hepatitis B Virus.

Definitions

For convenience, the meaning of certain terms and phrases used in the specification,

examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A", "U" and "T" or "dT" respectively, each generally stand for a nucleotide that contains guanine, cytosine, adenine, uracil and deoxythymidine as a base, respectively. However, the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. Sequences comprising such replacement moieties are embodiments of the invention. As detailed below, the herein described dsRNA molecules may also comprise "overhangs", i.e. unpaired, overhanging nucleotides which are not directly involved in the RNA double helical structure normally formed by the herein defined pair of "sense strand" and "antisense strand". Often, such an overhanging stretch comprises the deoxythymidine nucleotide, in most embodiments, two deoxythymidines in the 3′ end. Such overhangs will be described and illustrated below.

The term "Hepatitis B Virus gene" as used herein relates to the genes necessary for replication and pathogenesis of Hepatitis B Virus, in particular to the genes that encode core protein, viral polymerase, surface antigen, e-antigen and the X protein and the genes that encode the functional fragments of the same. The term "Hepatitis B Virus gene/sequence" does not only relate to (the) wild-type sequence(s) but also to mutations and alterations which may be comprised in said gene/sequence. Accordingly, the present invention is not limited to the specific dsRNA molecules provided herein. The invention also relates to dsRNA molecules that comprise an antisense strand that is at least 85% complementary to the corresponding nucleotide stretch of an RNA transcript of a Hepatitis B Virus gene that comprises such mutations/alterations.

As used herein, the term "consensus sequence" refers to at least 13 contiguous nucleotides, preferably at least 17 contiguous nucleotides, most preferably at least 19 contiguous nucleotides, which is highly conserved among the Hepatitis B Virus genomic sequences of genotype A, B, C and D.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of a Hepatitis B Virus gene,

including mRNA that is a product of RNA processing of a primary transcription product.

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature. However, as detailed herein, such a "strand comprising a sequence" may also comprise modifications, like modified nucleotides.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence. "Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

Sequences referred to as "fully complementary" comprise base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence.

However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but preferably not more than 13 mismatched base pairs upon hybridization.

The terms "complementary", "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

The term "double-stranded RNA", "dsRNA molecule", or "dsRNA", as used herein, refers to a ribonucleic acid molecule, or complex of ribonucleic acid molecules, having a duplex

structure comprising two anti-parallel and substantially complementary nucleic acid strands. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3′-end of one strand and the 5′ end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3′-end of one strand and the 5′ end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs. The nucleotides in said "overhangs" may comprise between 0 and 5 nucleotides, whereby "0" means no additional nucleotide(s) that form(s) an "overhang" and whereas "5" means five additional nucleotides on the individual strands of the dsRNA duplex. These optional "overhangs" are located in the 3′ end of the individual strands. As will be detailed below, also dsRNA molecules which comprise only an "overhang" in one of the two strands may be useful and even advantageous in context of this invention. The "overhang" comprises preferably between 0 and 2 nucleotides. Most preferably two "dT" (deoxythymidine) nucleotides are found at the 3′ end of both strands of the dsRNA. Also two "U"(uracil) nucleotides can be used as overhangs at the 3′ end of both strands of the dsRNA. Accordingly, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3′-end of one strand of the dsRNA extends beyond the 5′-end of the other strand, or vice versa. For example the antisense strand comprises 23 nucleotides and the sense strand comprises 21 nucleotides, forming a two nucleotide overhang at the 3′ end of the antisense strand. Preferably, the two nucleotide overhang is fully complementary to the mRNA of the target gene. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence. Where the region of

complementarity is not fully complementary to the target sequence, the mismatches are most tolerated outside nucleotides 2-7 of the 5′ terminus of the antisense strand

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand. "Substantially complementary" means preferably at least 85% of the overlapping nucleotides in sense and antisense strand are complementary.

"Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells *in vitro*; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, dsRNA can be injected into a tissue site or administered systemically. It is, for example envisaged that the dsRNA molecules of this invention be administered to a subject in need of medical intervention. Such an administration may comprise the injection of the dsRNA, the vector or a cell of this invention into a diseased site in said subject, for example into liver tissue/cells or into cancerous tissues/cells, like liver cancer tissue. In addition, the injection is preferably in close proximity to the diseased tissue envisaged. *In vitro* introduction into a cell includes methods known in the art such as electroporation and lipofection.

As used herein, "chronic liver diseases/disorders" refers to the functional abnormality of liver lasting more than six months which can be caused by the infection of virus. One example of the chronic liver diseases/disorders is chronic hepatitis (CH).

The term "inflammation" as used herein refers to the biologic response of body tissue to injury, irritation, or disease which can be caused by harmful stimuli, for example, pathogens, damaged cells, or irritants. Inflammation is typically characterized by pain and swelling. Inflammation is intended to encompass both acute responses, in which inflammatory processes are active (e.g., neutrophils and leukocytes), and chronic responses, which are marked by slow progress, a shift in the type of cell present at the site of inflammation, and the formation of connective tissue. One example of an inflammation-

caused disease is fibrosis.

The term "fibrotic conditions" as used herein refers to the functional problem of organs which can be caused by growth of fibrous tissue. One such example of such kind of disease is hepatic cirrhosis (HC).

The term "proliferating" and "proliferation" as used herein refer to cells undergoing mitosis. Throughout this application, the term "proliferative disorder" refers to any disease/disorder marked by unwanted or aberrant proliferation of tissue. As used herein, the term "proliferative disorder" also refers to conditions in which the unregulated and/or abnormal growth of cells can lead to the development of an unwanted condition or disease, which can be cancerous or non-cancerous.

Cancers to be treated comprise, but are again not limited to liver cancer, whereby said liver cancer may, inter alia, be selected from the group consisting of hepatocellular carcinoma (HCC), hepatoblastoma, a mixed liver cancer, a cancer derived from mesenchymal tissue, a liver sarcoma or a cholangiocarcinoma.

The terms "silence", "inhibit the expression of" and "knock down", in as far as they refer to a Hepatitis B Virus gene, herein refer to the at least partial suppression of the expression of a Hepatitis B Virus gene, as manifested by a reduction of the amount of mRNA transcribed from a Hepatitis B Virus gene which may be isolated from a first cell or group of cells in which a Hepatitis B Virus gene is transcribed and which has or have been treated such that the expression of a Hepatitis B Virus gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} \times 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to the Hepatitis B Virus gene transcription, e.g. the amount of protein encoded by a Hepatitis B Virus gene which is secreted by a cell, or the number of

cells displaying a certain phenotype.

As illustrated in the appended examples and in the appended Tables provided herein, the inventive dsRNA molecules are capable of inhibiting the expression of a Hepatitis B Virus by at least about 60%, preferably by at least 70%, most preferably by at least 80% in *in vitro* assays, i.e. *in vitro*. The term "*in vitro*" as used herein includes but is not limited to cell culture assays. The person skilled in the art can readily determine such an inhibition rate and related effects, in particular in light of the assays provided herein.

The term "off target" as used herein refers to all non-target mRNAs of the transcriptome that are predicted by *in silico* methods to hybridize to the described dsRNAs based on sequence complementarity. The dsRNAs of the present invention preferably do specifically inhibit the expression of Hepatitis B Virus gene, i.e. do not inhibit the expression of any off-target.

The term "half-life" as used herein is a measure of stability of a compound or molecule and can be assessed by methods known to a person skilled in the art, especially in light of the assays provided herein.

The term "non-immunostimulatory" as used herein refers to the absence of any induction of an immune response by the invented dsRNA molecules. Methods to determine immune responses are well known to a person skilled in the art, for example by assessing the release of cytokines, as described in the examples section.

The terms "treat", "treatment", and the like, mean in context of this invention the relief from or alleviation of a disorder related to Hepatitis B Virus infection, like chronic liver diseases/disorders, inflammations, fibrotic conditions and proliferative disorders, like cancers.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of at least one kind of dsRNAs and a pharmaceutically acceptable carrier. However, such a "pharmaceutical composition" may also comprise individual strands of such dsRNA molecules or the herein described vector(s) comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a sense or an

antisense strand comprised in the dsRNAs of this invention. It is also envisaged that cells, tissues or isolated organs that express or comprise the herein defined dsRNAs may be used as "pharmaceutical compositions". As used herein, "pharmacologically effective amount," "therapeutically effective amount," or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives as known to persons skilled in the art.

It is in particular envisaged that the pharmaceutically acceptable carrier allows for the systemic administration of the dsRNAs, vectors or cells of this invention. Whereas also the enteric administration is envisaged the parenteral administration and also transdermal or transmucosal (e.g. insufflation, buccal, vaginal, anal) administration as well as inhalation of the drug are feasible ways of administering to a patient in need of medical intervention the compounds of this invention. When parenteral administration is employed, this can comprise the direct injection of the compounds of this invention into the diseased tissue or at least in close proximity. However, also intravenous, intraarterial, subcutaneous, intramuscular, intraperitoneal, intradermal, intrathecal and other administrations of the compounds of this invention are within the skill of the artisan, for example the attending physician.

For intramuscular, subcutaneous and intravenous use, the pharmaceutical compositions of the invention will generally be provided in sterile aqueous solutions or suspensions, buffered to an appropriate pH and isotonicity. In a preferred embodiment, the carrier consists exclusively of an aqueous buffer. In this context, "exclusively" means no auxiliary agents or encapsulating substances are present which might affect or mediate uptake of dsRNA in the cells that express a Hepatitis B Virus gene. Aqueous suspensions according to the invention may include suspending agents such as cellulose derivatives, sodium

alginate, polyvinyl-pyrrolidone and gum tragacanth, and a wetting agent such as lecithin. Suitable preservatives for aqueous suspensions include ethyl and n-propyl p-hydroxybenzoate. The pharmaceutical compositions useful according to the invention also include encapsulated formulations to protect the dsRNA against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. Liposomal suspensions and bi-specific antibodies can also be used as pharmaceutically acceptable carriers. These can be prepared according to methods known to those skilled in the art, for example, as described in PCT publication WO91/06309 and WO2011/003780.

As used herein, a "transformed cell" is a cell into which at least one vector has been introduced from which a dsRNA molecule or at least one strand of such a dsRNA molecule may be expressed. Such a vector is preferably a vector comprising a regulatory sequence operably linked to nucleotide sequence that encodes at least one sense strand or antisense strand of a dsRNA of the present invention.

It can be reasonably expected that shorter dsRNAs comprising one of the sequences in Table 1 and 4 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above.

In one preferred embodiment the inventive dsRNA molecules comprise nucleotides 1-19 of the sequences given in Table 1.

As pointed out above, in most embodiments of this invention, the dsRNA molecules provided herein comprise a duplex length (i.e. without "overhangs") of about 16 to about 30 nucleotides. Particular useful dsRNA duplex lengths are about 19 to about 25 nucleotides. Most preferred are duplex structures with a length of 19 nucleotides. In the inventive dsRNA molecules, the antisense strand is at least partially complementary to the sense strand.

The dsRNA of the invention can contain one or more mismatches to the target sequence.

In a preferred embodiment, the dsRNA of the invention contains no more than 13 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located within nucleotides 2-7 of the 5′ terminus of the antisense strand. In another embodiment it is preferable that the area of mismatch not be located within nucleotides 2-9 of the 5′ terminus of the antisense strand.

As mentioned above, at least one end/strand of the dsRNA may have a single-stranded nucleotide overhang of 1 to 5, preferably 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the dsRNA, without affecting its overall stability. dsRNA having only one overhang has proven particularly stable and effective *in vivo*, as well as in a variety of cells, cell culture mediums, blood, and serum. Preferably, the single-stranded overhang is located at the 3′-terminal end of the antisense strand or, alternatively, at the 3′-terminal end of the sense strand. The dsRNA may also have a blunt end, preferably located at the 5′-end of the antisense strand. Preferably, the antisense strand of the dsRNA has a nucleotide overhang at the 3′-end, and the 5′-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

The dsRNA of the present invention may also be chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art. Chemical modifications may include, but are not limited to 2′ modifications, introduction of non-natural bases, covalent attachment to a ligand, and replacement of phosphate linkages with thiophosphate linkages, inverted deoxythymidines. In this embodiment, the integrity of the duplex structure is strengthened by at least one, and preferably two, chemical linkages. Chemical linking may be achieved by any of a variety of well-known techniques, for example by introducing covalent, ionic or hydrogen bonds; hydrophobic interactions, van der Waals or stacking interactions; by means of metal-ion coordination, or through use of purine analogues. Preferably, the chemical groups that can be used to modify the dsRNA include, without limitation, methylene blue; bifunctional groups, preferably bis-(2-chloroethyl)amine; N-acetyl-N'-(p-glyoxylbenzoyl)cystamine; 4-thiouracil; and psoralen. In one preferred embodiment, the

linker is a hexa-ethylene glycol linker. In this case, the dsRNA are produced by solid phase synthesis and the hexa-ethylene glycol linker is incorporated according to standard methods (e.g., Williams DJ and Hall KB, *Biochem.* (1996) 35:14665-14670). In a particular embodiment, the 5′-end of the antisense strand and the 3′-end of the sense strand are chemically linked via a hexaethylene glycol linker. In another embodiment, at least one nucleotide of the dsRNA comprises a phosphorothioate or phosphorodithioate groups. The chemical bond at the ends of the dsRNA is preferably formed by triple-helix bonds.

In certain embodiments, a chemical bond may be formed by means of one or several bonding groups, wherein such bonding groups are preferably poly-(oxyphosphinicooxy-1,3-propandiol) and/or polyethylene glycol chains. In other embodiments, a chemical bond may also be formed by means of purine analogs introduced into the double-stranded structure instead of purines. In further embodiments, a chemical bond may be formed by azabenzene units introduced into the double-stranded structure. In still further embodiments, a chemical bond may be formed by branched nucleotide analogs instead of nucleotides introduced into the double- stranded structure. In certain embodiments, a chemical bond may be induced by ultraviolet light.

In yet another embodiment, the nucleotides at one or both of the two single strands may be modified to prevent or inhibit the activation of cellular enzymes, for example certain nucleases. Techniques for inhibiting the activation of cellular enzymes are known in the art including, but not limited to, 2′-amino modifications, 2′-amino sugar modifications, 2′-F sugar modifications, 2′-F modifications, 2′-alkyl sugar modifications, uncharged backbone modifications, morpholino modifications, 2′-O-methyl modifications, and phosphoramidate (see, e.g., Wagner, *Nat. Med.* (1995) 1:1116-8). Thus, at least one 2′-hydroxyl group of the nucleotides on a dsRNA is replaced by a chemical group, preferably by a 2′-amino or a 2′-methyl group. Also, at least one nucleotide may be modified to form a locked nucleotide. Such locked nucleotide contains a methylene bridge that connects the 2′-oxygen of ribose with the 4′- carbon of ribose. Introduction of a locked nucleotide into an oligonucleotide improves the affinity for complementary sequences and increases the melting temperature by several degrees.

Modifications of dsRNA molecules provided herein may positively influence their stability *in vivo* as well as *in vitro* and also improve their delivery to the (diseased) target

side. Furthermore, such structural and chemical modifications may positively influence physiological reactions towards the dsRNA molecules upon administration, e.g. the cytokine release which is preferably suppressed. Such chemical and structural modifications are known in the art and are, inter alia, illustrated in Nawrot *Current Topics in Med Chem*, (2006) 6:913-925.

Conjugating a ligand to a dsRNA can enhance its cellular absorption as well as targeting to a particular tissue. In certain instances, a hydrophobic ligand is conjugated to the dsRNA to facilitate direct permeation of the cellular membrane. Alternatively, the ligand conjugated to the dsRNA is a substrate for receptor-mediated endocytosis. These approaches have been used to facilitate cell permeation of antisense oligonucleotides. For example, cholesterol has been conjugated to various antisense oligonucleotides resulting in compounds that are substantially more active compared to their non-conjugated analogs (See Manoharan M, *Antisense & Nucleic Acid Drug Development* (2002) 12:103). Other lipophilic compounds that have been conjugated to oligonucleotides include 1-pyrene butyric acid, 1,3-bis-O-(hexadecyl)glycerol, and menthol. One example of a ligand for receptor-mediated endocytosis is folic acid. Folic acid enters the cell by folate-receptor-mediated endocytosis. dsRNA compounds bearing folic acid would be efficiently transported into the cell via the folate-receptor-mediated endocytosis. Attachment of folic acid to the 3′-terminus of an oligonucleotide results in increased cellular uptake of the oligonucleotide (Li S, Deshmukh HM, and Huang L, *Pharm. Res.* (1998) 15:1540). Other ligands that have been conjugated to oligonucleotides include polyethylene glycols, carbohydrate clusters, cross-linking agents, porphyrin conjugates, and delivery peptides.

In certain instances, conjugation of a cationic ligand to oligonucleotides often results in improved resistance to nucleases. Representative examples of cationic ligands are propylammonium and dimethylpropylammonium. Interestingly, antisense oligonucleotides were reported to retain their high binding affinity to mRNA when the cationic ligand was dispersed throughout the oligonucleotide. See Manoharan M, *Antisense & Nucleic Acid Drug Development* (2002) 12:103 and references therein.

The ligand-conjugated dsRNA of the invention may be synthesized by the use of a dsRNA that bears a pendant reactive functionality, such as that derived from the attachment of a linking molecule onto the dsRNA. This reactive oligonucleotide may be reacted directly

with commercially-available ligands, ligands that are synthesized bearing any of a variety of protecting groups, or ligands that have a linking moiety attached thereto. The methods of the invention facilitate the synthesis of ligand-conjugated dsRNA by the use of, in some preferred embodiments, nucleoside monomers that have been appropriately conjugated with ligands and that may further be attached to a solid-support material. Such ligand-nucleoside conjugates, optionally attached to a solid-support material, are prepared according to some preferred embodiments of the methods of the invention via reaction of a selected serum-binding ligand with a linking moiety located on the 5′ position of a nucleoside or oligonucleotide. In certain instances, a dsRNA bearing an aralkyl ligand attached to the 3′-terminus of the dsRNA is prepared by first covalently attaching a monomer building block to a controlled-pore-glass support via a long-chain aminoalkyl group. Then, nucleotides are bonded via standard solid-phase synthesis techniques to the monomer building-block bound to the solid support. The monomer building block may be a nucleoside or other organic compound that is compatible with solid-phase synthesis.

The dsRNA used in the conjugates of the invention may be conveniently and routinely made through the well-known technique of solid-phase synthesis. It is also known to use similar techniques to prepare other oligonucleotides, such as the phosphorothioates and alkylated derivatives.

Teachings regarding the synthesis of particular modified oligonucleotides may be found in the following U.S. patents: U.S. Pat. No. 5,218,105, drawn to polyamine conjugated oligonucleotides; U.S. Pat. Nos. 5,541,307, drawn to oligonucleotides having modified backbones; U.S. Pat. No. 5,521,302, drawn to processes for preparing oligonucleotides having chiral phosphorus linkages; U.S. Pat. No. 5,539,082, drawn to peptide nucleic acids; U.S. Pat. No. 5,554,746, drawn to oligonucleotides having β-lactam backbones; U.S. Pat. No. 5,571,902, drawn to methods and materials for the synthesis of oligonucleotides; U.S. Pat. No. 5,578,718, drawn to nucleosides having alkylthio groups, wherein such groups may be used as linkers to other moieties attached at any of a variety of positions of the nucleoside; U.S. Pat. No 5,587,361 drawn to oligonucleotides having phosphorothioate linkages of high chiral purity; U.S. Pat. No. 5,506,351, drawn to processes for the preparation of 2′-O-alkyl guanosine and related compounds, including 2,6-diaminopurine compounds; U.S. Pat. No. 5,587,469, drawn to oligonucleotides having N-2 substituted purines; U.S. Pat. No. 5,587,470, drawn to oligonucleotides having

3-deazapurines; U.S. Pat. No. 5,608,046, both drawn to conjugated 4′-desmethyl nucleoside analogs; U.S. Pat. No. 5,610,289, drawn to backbone-modified oligonucleotide analogs; U.S. Pat. No 6,262,241 drawn to, inter alia, methods of synthesizing 2′-fluoro-oligonucleotides.

In the ligand-conjugated dsRNA and ligand-molecule bearing sequence-specific linked nucleosides of the invention, the oligonucleotides and oligonucleosides may be assembled on a suitable oligonucleotide synthesizer utilizing standard nucleotide or nucleoside precursors, or nucleotide or nucleoside conjugate precursors that already bear the linking moiety, ligand-nucleotide or nucleoside-conjugate precursors that already bear the ligand molecule, or non-nucleoside ligand-bearing building blocks.

When using nucleotide-conjugate precursors that already bear a linking moiety, the synthesis of the sequence-specific linked nucleosides is typically completed, and the ligand molecule is then reacted with the linking moiety to form the ligand-conjugated oligonucleotide. Oligonucleotide conjugates bearing a variety of molecules such as steroids, vitamins, lipids and reporter molecules, has previously been described (see Manoharan et al., PCT Application WO 93/07883). In a preferred embodiment, the oligonucleotides or linked nucleosides of the invention are synthesized by an automated synthesizer using phosphoramidites derived from ligand-nucleoside conjugates in addition to commercially available phosphoramidites.

The incorporation of a 2′-O-methyl, 2′-O-ethyl, 2′-O-propyl, 2′-O-allyl, 2′-O-aminoalkyl or 2′-deoxy-2′-fluoro group in nucleosides of an oligonucleotide confers enhanced hybridization properties to the oligonucleotide. Further, oligonucleotides containing phosphorothioate backbones have enhanced nuclease stability. Thus, functionalized, linked nucleosides of the invention can be augmented to include either or both a phosphorothioate backbone or a 2′-O-methyl, 2′-O-ethyl, 2′-O-propyl, 2′-O-aminoalkyl, 2′-O-allyl or 2′-deoxy-2′-fluoro group.

In some preferred embodiments, functionalized nucleoside sequences of the invention possessing an amino group at the 5′-terminus are prepared using a DNA synthesizer, and then reacted with an active ester derivative of a selected ligand. Active ester derivatives are well known to those skilled in the art. Representative active esters include

N-hydrosuccinimide esters, tetrafluorophenolic esters, pentafluorophenolic esters and pentachlorophenolic esters. The reaction of the amino group and the active ester produces an oligonucleotide in which the selected ligand is attached to the 5′-position through a linking group. The amino group at the 5′- terminus can be prepared utilizing a 5′-Amino-Modifier C6 reagent. In a preferred embodiment, ligand molecules may be conjugated to oligonucleotides at the 5′-position by the use of a ligand-nucleoside phosphoramidite wherein the ligand is linked to the 5′-hydroxy group directly or indirectly via a linker. Such ligand-nucleoside phosphoramidites are typically used at the end of an automated synthesis procedure to provide a ligand-conjugated oligonucleotide bearing the ligand at the 5′-terminus.

In one preferred embodiment of the methods of the invention, the preparation of ligand conjugated oligonucleotides commences with the selection of appropriate precursor molecules upon which to construct the ligand molecule. Typically, the precursor is an appropriately- protected derivative of the commonly-used nucleosides. For example, the synthetic precursors for the synthesis of the ligand-conjugated oligonucleotides of the invention include, but are not limited to, 2′-aminoalkoxy-5′-ODMT-nucleosides, 2′-6-aminoalkylamino-5′-ODMT-nucleosides, 5′-6-aminoalkoxy-2′-deoxy-nucleosides, 5′-6-aminoalkoxy-2-protected-nucleosides, 3′-6- aminoalkoxy-5′-ODMT-nucleosides, and 3′-aminoalkylamino-5′-ODMT-nucleosides that may be protected in the nucleobase portion of the molecule. Methods for the synthesis of such amino-linked protected nucleoside precursors are known to those of ordinary skill in the art.

In many cases, protecting groups are used during the preparation of the compounds of the invention. As used herein, the term "protected" means that the indicated moiety has a protecting group appended thereon. In some preferred embodiments of the invention, compounds contain one or more protecting groups. A wide variety of protecting groups can be employed in the methods of the invention. In general, protecting groups render chemical functionalities inert to specific reaction conditions, and can be appended to and removed from such functionalities in a molecule without substantially damaging the remainder of the molecule.

Protecting groups in general and hydroxyl protecting groups in particular are well known in the art (Greene and Wuts, *Protective Groups in Organic Synthesis*, Chapter 2, 2d ed.,

John Wiley & Sons, New York, 1991). Amino-protecting groups stable to acid treatment are selectively removed with base treatment, and are used to make reactive amino groups selectively available for substitution. Examples of such groups are the Fmoc and various substituted sulfonylethyl carbamates exemplified by the Nsc group.

Additional amino-protecting groups include, but are not limited to, carbamate protecting groups, such as 2-trimethylsilylethoxycarbonyl (Teoc), 1-methyl-1-(4-biphenylyl)-ethoxycarbonyl (Bpoc), t-butoxycarbonyl (BOC), allyloxycarbonyl (Alloc), 9-fluorenyl-methyloxycarbonyl (Fmoc), and benzyloxycarbonyl (Cbz); amide protecting groups, such as formyl, acetyl, trihaloacetyl, benzoyl, and nitrophenylacetyl; sulfonamide protecting groups, such as 2-nitrobenzenesulfonyl; and imine and cyclic imide protecting groups, such as phthalimido and dithiasuccinoyl. Equivalents of these amino-protecting groups are also encompassed by the compounds and methods of the invention.

Many solid supports are commercially available and one of ordinary skill in the art can readily select a solid support to be used in the solid-phase synthesis steps. In certain embodiments, a universal support is used. A universal support, well known in the art, allows for the preparation of oligonucleotides having unusual or modified nucleotides located at the 3′-terminus of the oligonucleotide. In addition, it has been reported that the oligonucleotide can be cleaved from the universal support under milder reaction conditions when the oligonucleotide is bonded to the solid support via a syn-1,2-acetoxyphosphate group which more readily undergoes basic hydrolysis. See Guzaev AI, and Manoharan MJ. *Am. Chem. Soc.* (2003) 125:2380.

The nucleosides are linked by phosphorus-containing or non-phosphorus-containing covalent internucleoside linkages. For the purposes of identification, such conjugated nucleosides can be characterized as ligand-bearing nucleosides or ligand-nucleoside conjugates. The linked nucleosides having an aralkyl ligand conjugated to a nucleoside within their sequence will demonstrate enhanced dsRNA activity when compared to like dsRNA compounds that are not conjugated.

The aralkyl-ligand-conjugated oligonucleotides of the invention also include conjugates of oligonucleotides and linked nucleosides wherein the ligand is attached directly to the nucleoside or nucleotide without the intermediacy of a linker group. The ligand may

preferably be attached, via linking groups, at a carboxyl, amino or oxo group of the ligand. Typical linking groups may be ester, amide or carbamate groups.

Specific examples of preferred modified oligonucleotides envisioned for use in the ligand-conjugated oligonucleotides of the invention include oligonucleotides containing modified backbones or non-natural internucleoside linkages. As defined here, oligonucleotides having modified backbones or internucleoside linkages include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of the invention, modified oligonucleotides that do not have a phosphorus atom in their intersugar backbone can also be considered to be oligonucleosides.

Specific oligonucleotide chemical modifications are described below. It is not necessary for all positions in a given compound to be uniformly modified. Conversely, more than one modifications may be incorporated in a single dsRNA compound or even in a single nucleotide thereof.

Preferred modified internucleoside linkages or backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3′-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates including 3′-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3′-5′ linkages, 2′-5′ linked analogs of these, and those having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3′-5′ to 5′-3′ or 2′-5′ to 5′-2′. Various salts, mixed salts and free-acid forms are also included.

Representative United States Patents relating to the preparation of the above phosphorus-atom-containing linkages include, but are not limited to, U.S. Pat. Nos. 4,469,863, 5,023,243, 5,264,423, 5,321,131, 5,399,676, 5,405,939, 5,453,496, 5,455,233, and 5,466,677.

Preferred modified internucleoside linkages or backbones that do not include a phosphorus atom therein (i.e., oligonucleosides) have backbones that are formed by short chain alkyl

or cycloalkyl intersugar linkages, mixed heteroatom and alkyl or cycloalkyl intersugar linkages, or one or more short chain heteroatomic or heterocyclic intersugar linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts.

Representative United States patents relating to the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506, 5,214,134, 5,216,141, 5,264,562, 5,466,677, 5,470,967, 5,489,677, 5,602,240, and 5,663,312.

In other preferred oligonucleotide mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleoside units are replaced with novel groups. The nucleobase units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligonucleotide, an oligonucleotide mimetic, that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar-backbone of an oligonucleotide is replaced with an amide-containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to atoms of the amide portion of the backbone. Teaching of PNA compounds can be found for example in U.S. Pat. No. 5,539,082.

Some preferred embodiments of the invention employ oligonucleotides with phosphorothioate linkages and oligonucleosides with heteroatom backbones, and in particular $-CH_2-NH-O-CH_2-$, $-CH_2-N(CH_3)-O-CH_2-$ [known as a methylene (methylimino) or MMI backbone], $-CH_2-O-N(CH_3)-CH_2-$, $-CH_2-N(CH_3)-N(CH_3)-CH_2-$, and $-O-N(CH_3)-CH_2-CH_2-$ [wherein the native phosphodiester backbone is represented as $-O-P-O-CH_2-$] of the above referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above referenced U.S. Pat. No. 5,602,240. Also preferred are oligonucleotides having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

The oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C), and uracil (U). Modified nucleobases include other synthetic and natural nucleobases, such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2- thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8- hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5- trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine.

Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, or otherwise known in the art or commercially available. Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligonucleotides of the invention. These include 5-substituted pyrimidines, 6- azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-Methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2°C and are presently preferred base substitutions, even more particularly when combined with 2′-methoxyethyl sugar modifications.

Representative United States patents relating to the preparation of certain of the above-noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 5,134,066, 5,459,255, 5,552,540, 5,594,121, and 5,596,091.

In certain embodiments, the oligonucleotides employed in the ligand-conjugated oligonucleotides of the invention may additionally or alternatively comprise one or more

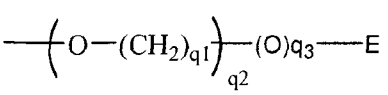
substituted sugar moieties. Preferred oligonucleotides comprise one of the following at the 2′ position: OH; F; O-, S-, or N-alkyl, O-, S-, or N-alkenyl, or O, S- or N-alkynyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C_1 to C_{10} alkyl or C_2 to C_{10} alkenyl and alkynyl. Particularly preferred are $O[(CH_2)_nO]_mCH_3$, $O(CH_2)_nOCH_3$, $O(CH_2)_nNH_2$, $O(CH_2)_nCH_3$, $O(CH_2)_nONH_2$, and $O(CH_2)_nON[(CH_2)_nCH_3)]_2$, where n and m are from 1 to about 10. Other preferred oligonucleotides comprise one of the following at the 2′ position: C1 to C10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. A preferred modification includes 2′-methoxyethoxy (2′-O−$CH_2CH_2OCH_3$, also known as 2′-O-(2-methoxyethyl) or 2′-MOE), i.e., an alkoxyalkoxy group. A further preferred modification includes 2′-dimethylaminooxyethoxy, i.e., a $O(CH_2)_2ON(CH_3)_2$ group, also known as 2′-DMAOE, as described in U.S. Pat. No. 6,127,533.

Other preferred modifications include 2′-methoxy (2′-O−CH_3), 2′-aminopropoxy (2′-$OCH_2CH_2CH_2NH_2$) and 2′-fluoro (2′-F). Similar modifications may also be made at other positions on the oligonucleotide, particularly the 3′ position of the sugar on the 3′ terminal nucleotide or in 2′-5′ linked oligonucleotides.

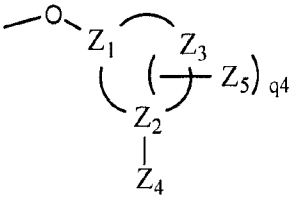
As used herein, the term ..sugar substituent group" or "2′-substituent group" includes groups attached to the 2′-position of the ribofuranosyl moiety with or without an oxygen atom. Sugar substituent groups include, but are not limited to, fluoro, O-alkyl, O-alkylamino, O-alkylalkoxy, protected O-alkylamino, O-alkylaminoalkyl, O-alkyl imidazole and polyethers of the formula (O-alkyl)m, wherein m is 1 to about 10. Preferred among these polyethers are linear and cyclic polyethylene glycols (PEGs), and (PEG)-containing groups, such as crown ethers and, inter alia, those which are disclosed by Delgardo et. al. (*Critical Reviews in Therapeutic Drug Carrier Systems* (1992) 9:249). Further sugar modifications are disclosed by Cook (*Anti-fibrosis Drug Design*, (1991) 6:585-607). Fluoro, O-alkyl, O-alkylamino, O-alkyl imidazole, O-alkylaminoalkyl, and alkyl amino substitution is described in U.S. Patent 6,166,197, entitled "Oligomeric

Compounds having Pyrimidine Nucleotide(s) with 2′ and 5′ Substitutions."

Additional sugar substituent groups amenable to the invention include 2′-SR and 2′-NR_2 groups, wherein each R is, independently, hydrogen, a protecting group or substituted or unsubstituted alkyl, alkenyl, or alkynyl. 2′-SR Nucleosides are disclosed in U.S. Pat. No. 5,670,633. The incorporation of 2′-SR monomer synthons is disclosed by Hamm et al. (*J. Org. Chem.*, (1997) 62:3415-3420). 2′-NR nucleosides are disclosed by Thomson JB, *J. Org. Chem.*, (1996) 61:6273-6281; and Polushin et al., *Tetrahedron Lett.*, (1996) 37:3227-3230. Further representative 2′-substituent groups amenable to the invention include those having one of formula I or II:



I



II

wherein

E is C_1-C_{10} alkyl, N(Q3)(Q4) or N=C(Q3)(Q4); each Q3 and Q4 is, independently, H, C_1-C_{10} alkyl, dialkylaminoalkyl, a nitrogen protecting group, a tethered or untethered conjugate group, a linker to a solid support; or Q3 and Q4, together, form a nitrogen protecting group or a ring structure optionally including at least one additional heteroatom selected from N and O;

q1 is an integer from 1 to 10;

q2 is an integer from 1 to 10;

q3 is 0 or 1;

q4 is 0, 1 or 2;

each Z1, Z2, and Z3 is, independently, C_4-C_7 cycloalkyl, C_5-C_{14} aryl or C_3-C_{15} heterocyclyl, wherein the heteroatom in said heterocyclyl group is selected from oxygen, nitrogen and sulfur;

Z4 is OM1, SM1, or $N(M1)_2$; each M1 is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, C(=NH)N(H)M2, C(=O)N(H)M2 or OC(=O)N(H)M2; M2 is H or C_1-C_8 alkyl; and

Z5 is C_1-C_{10} alkyl, C_1-C_{10} haloalkyl, C_2-C_{10} alkenyl, C_2-C_{10} alkynyl, C_6-C_{14} aryl, N(Q3)(Q4), OQ3, halo, SQ3 or CN.

Representative 2'-O-sugar substituent groups of formula I are disclosed in U.S. Pat. No. 6,172,209, entitled "Capped 2'-Oxyethoxy Oligonucleotides". Representative cyclic 2'-O-sugar substituent groups of formula II are disclosed in U.S. Patent 6,271,358, entitled "RNA Targeted 2'-Modified Oligonucleotides that are Conformationally Preorganized".

Sugars having O-substitutions on the ribosyl ring are also amenable to the invention. Representative substitutions for ring O include, but are not limited to, S, CH_2, CHF, and CF_2.

Oligonucleotides may also have sugar mimetics, such as cyclobutyl moieties, in place of the pentofuranosyl sugar. Representative United States patents relating to the preparation of such modified sugars include, but are not limited to, U.S. Pat. Nos. 5,359,044, 5,466,786, 5,519,134, 5,591,722, 5,597,909, 5,646,265, and 5,700,920.

Additional modifications may also be made at other positions on the oligonucleotide, particularly the 3' position of the sugar on the 3' terminal nucleotide. For example, one additional modification of the ligand-conjugated oligonucleotides of the invention involves chemically linking to the oligonucleotide one or more additional non-ligand moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the oligonucleotide. Such moieties include but are not limited to lipid moieties, such as a cholesterol moiety (Letsinger et al., *Proc. Natl. Acad. Sci.* USA, (1989) 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, (1994) 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, (1992) 660:306; Manoharan et al., *Bioorg. Med. Chem. Let.*, (1993) 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, (1992) 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, (1991) 10:111; Kabanov et al., *FEBS Lett.*, (1990) 259:327; Svinarchuk et al., *Biochimie*, (1993) 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, (1995) 36:3651; Shea et al., *Nucl. Acids Res.*, (1990) 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides &*

Nucleotides, (1995) 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, (1995) 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, (1995) 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, (1996) 277:923).

The invention also includes compositions employing oligonucleotides that are substantially chirally pure with regard to particular positions within the oligonucleotides. Examples of substantially chirally pure oligonucleotides include, but are not limited to, those having phosphorothioate linkages that are at least 75% Sp or Rp (Cook et al., U.S. Pat. No. 5,587,361) and those having substantially chirally pure (Sp or Rp) alkylphosphonate, phosphoramidate or phosphotriester linkages (Cook, U.S. Pat. Nos. 5,212,295 and 5,521,302).

In certain instances, the oligonucleotide may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to oligonucleotides in order to enhance the activity, cellular distribution or cellular uptake of the oligonucleotide, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, (1989, 86:6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, (1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, (1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., (1993, 3:2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, (1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, (1991) 10:111; Kabanov et al., *FEBS Lett.*, (1990) 259:327; Svinarchuk et al., *Biochimie*, (1993) 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, (1995) 36:3651; Shea et al., *Nucl. Acids Res.*, (1990) 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, (1995) 14:969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, (1995) 36:3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, (1995) 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, (1996) 277:923). Typical conjugation protocols involve the synthesis of oligonucleotides bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating

reagents. The conjugation reaction may be performed either with the oligonucleotide still bound to the solid support or following cleavage of the oligonucleotide in solution phase. Purification of the oligonucleotide conjugate by HPLC typically affords the pure conjugate.

Alternatively, the molecule being conjugated may be converted into a building block, such as a phosphoramidite, via an alcohol group present in the molecule or by attachment of a linker bearing an alcohol group that may be phosphorylated.

Importantly, each of these approaches may be used for the synthesis of ligand conjugated oligonucleotides. Amino linked oligonucleotides may be coupled directly with ligand via the use of coupling reagents or following activation of the ligand as an NHS or pentfluorophenolate ester. Ligand phosphoramidites may be synthesized via the attachment of an aminohexanol linker to one of the carboxyl groups followed by phosphitylation of the terminal alcohol functionality. Other linkers, such as cysteamine, may also be utilized for conjugation to a chloroacetyl linker present on a synthesized oligonucleotide.

The person skilled in the art is readily aware of methods to introduce the molecules of this invention into cells, tissues or organisms. Corresponding examples have also been provided in the detailed description of the invention above. For example, the nucleic acid molecules or the vectors of this invention, encoding for at least one strand of the inventive dsRNAs may be introduced into cells or tissues by methods known in the art, like transfections etc.

Also for the introduction of dsRNA molecules, means and methods have been provided. For example, targeted delivery by glycosylated and folate-modified molecules, including the use of polymeric carriers with ligands, such as galactose and lactose or the attachment of folic acid to various macromolecules allows the binding of molecules to be delivered to folate receptors. Targeted delivery by peptides and proteins other than antibodies, for example, including RGD-modified nanoparticles to deliver siRNA *in vivo* or multicomponent (nonviral) delivery systems including short cyclodextrins, adamantine-PEG are known. Yet, also the targeted delivery using antibodies or antibody fragments, including (monovalent) Fab-fragments of an antibody (or other fragments of such an

antibody) or single-chain antibodies are envisaged. Injection approaches for target directed delivery comprise, inter alia, hydrodynamic i.v. injection. Also cholesterol conjugates of dsRNA may be used for targeted delivery, whereby the conjugation to lipohilic groups enhances cell uptake and improve pharmacokinetics and tissue biodistribution of oligonucleotides. Also cationic delivery systems are known, whereby synthetic vectors with net positive (cationic) charge to facilitate the complex formation with the polyanionic nucleic acid and interaction with the negatively charged cell membrane. Such cationic delivery systems comprise also cationic liposomal delivery systems, cationic polymer and peptide delivery systems. Other delivery systems for the cellular uptake of dsRNA/siRNA are aptamer-ds/siRNA. Also gene therapy approaches can be used to deliver the inventive dsRNA molecules or nucleic acid molecules encoding the same. Such systems comprise the use of non-pathogenic virus, modified viral vectors, as well as deliveries with nanoparticles or liposomes. Other delivery methods for the cellular uptake of dsRNA are extracorporeal, for example ex vivo treatments of cells, organs or tissues. Certain of these technologies are described and summarized in publications, like Akhtar, *Journal of Clinical Investigation* (2007) 117:3623-3632, Nguyen et al., *Current Opinion in Molecular Therapeutics* (2008) 10:158-167, Zamboni, *Clin Cancer Res* (2005) 11:8230-8234 or Ikeda et al., *Pharmaceutical Research* (2006) 23:1631-1640.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. In case of conflict with the publications, patent applications and patents referred to herein, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

The above provided embodiments and items of the present invention are now illustrated with the following, non-limiting examples.

EXAMPLES

Identification of dsRNAs for therapeutic use. dsRNA design was carried out to identify dsRNAs specifically targeting Hepatitis B Virus genotypes A, B, C and D for therapeutic

use.

First, the known Hepatitis B Virus genomic sequences were downloaded from NCBI Genbank (accessions listed in Table.6). The genotype information was either extracted form NCBI Genbank files or determined by computer aided comparison with reference genomes (accessions listed in Table. 6).

The Hepatitis B Virus genomic sequences of genotype A-D were examined by computer analysis to identify optimal target regions for RNAi agents, namely highly conserved 17 nucleotide long sequence stretches that were identical in at least 90% of all sequences.

In identifying RNAi agents, the selection was limited to 17mer sequences having at least two mismatches to any sequence in the human RefSeq database (release 41), which we assumed to represent the comprehensive human transcriptome, by using a proprietary algorithm.

All 17mer sequences containing four or more consecutive G's (poly-G sequences) were further excluded from the synthesis.

Sequences of 19 nucleotides length were defined that harbor the selected 17mers in position 2 to 18.

These 19mer sequences yield RNA interference (RNAi) agents cross-reactive to Hepatitis B Virus genomic sequences of genotype A-D and formed the basis for the synthesis of the RNAi agents in appended Tables 1 and 2.

dsRNA synthesis. Oligoribonucleotides were synthesized according to the phosphoramidite technology on solid phase. Depending on the scale either an ABI 394 synthesizer (Applied Biosystems) or an AKTA oligopilot 100 (GE Healthcare, Freiburg, Germany) was used. Syntheses were performed on a solid support made of controlled pore glass (CPG, 520Å, with a loading of 75 μmol/g, obtained from Prime Synthesis, Aston, PA, USA). All 2'-modified RNA phosphoramidites as well as ancillary reagents were purchased from SAFC (Hamburg, Germany). Specifically, the following 2'-O-Methyl phosphoramidites were used: (5'-O-dimethoxytrityl-N^6-(benzoyl)-2'-O-methyl-adenosine-3'-O-(2-cyanoethyl-

N,N-diisopropylamino) phosphoramidite, 5′-O- dimethoxytrityl-N^4-(acetyl)-2′-O-methyl-cytidine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, (5′-O-dimethoxy-trityl-N^2-(isobutyryl)-2′-O-methyl-guanosine-3′-O-(2-cyanoethyl-N,N-diisopropylamino) phosphoramidite, and 5′-O-dimethoxy-trityl-2′-O-methyl-uridine-3′-O-(2-cyanoethyl-N,N-diisopropylamino)phosphoramidite. The 2′-Deoxy-2′-fluoro-phosphoramidites carried the same protecting groups as the 2′-0-methyl RNA amidites. All amidites were dissolved in anhydrous acetonitrile (100 mM) and molecular sieves (3Å) were added. To generate the 5′-phosphate the 2-[2-(4,4′-Dimethoxytrityloxy) ethylsulfonyl]ethyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite from Glen Research (Sterling, Virginia, USA) was used. In order to introduce the C-6 aminolinker at the 5′-end of the oligomers the 6-(Trifluoroacetylamino)-hexyl-(2-cyanoethyl)-(N,N-diisopropyl)-phosphoramidite from Thermo Fisher Scientific (Milwaukee, Wisconsin, USA) was employed. The 5′-modifications were introduced without any modification of the synthesis cycle. 5-Ethyl thiotetrazole (ETT, 500 mM in acetonitrile) was used as activator solution. Coupling times were 6 min. In order to introduce phosphorothioate linkages, a 50 mM solution of 3-((Dimethylamino-methylidene)amino)-3H-1,2,4-dithiazole-3-thione (DDTT, obtained from AM Chemicals, Oceanside, CA, USA) in anhydrous Acetonitrile/pyridine (1:1 v/v) was employed.

Cleavage and deprotection of support bound oligomer. After finalization of the solid phase synthesis, cyanoethyl protecting groups were removed by a 30 min treatment with 20% Diethyl amine in ACN without cleaving the oligonucleotides from the support. Subsequently, the dried solid support was transferred to a 15 mL tube and treated with concentrated aqueous ammonia (Aldrich) for 18 h at 40°C. After centrifugation the supernatant was transferred to a new tube and the CPG was washed with aqueous ammonia. The combined solutions were evaporated and the solid residue was reconstituted in buffer A (see below).

Purification of oligoribonucleotides. Crude oligomers were purified by anionic exchange HPLC using a column packed with Source Q15 (GE Helthcare) and an AKTA Explorer system (GE Healthcare). Buffer A was 10 mM sodium perchlorate, 20 mM Tris, 1 mM EDTA, pH 7.4 (Fluka, Buchs, Switzerland) and contained 20% Acetonitrile and buffer B was the same as buffer A with the exception of 500 mM sodium perchlorate. A gradient of 22%B to 42%B within 32 column volumes (CV) was employed. UV traces at 280 nm

were recorded Appropriate fractions were pooled and precipitated with 3M NaOAc, pH=5.2 and 70% Ethanol. Finally, the pellet was washed with 70% Ethanol. Alternatively, desalting was carried out using Sephadex HiTrap columns (GE Helthcare) according to the manufacturer's recommendation.

Annealing of oligoribonucleotides to generate siRNA. Complementary strands were mixed by combining equimolar RNA solutions. The mixture was lyophilized and reconstituted with an appropriate volume of annealing buffer (100 mM NaCl, 20 mM sodium phosphate, pH 6.8) to achieve the desired concentration. This solution was placed into a water bath at 80°C which was cooled to RT within 3 h.

In Vitro *screening of HBV mRNA-targeting dsRNA.* The psiCHECK™-2 vector (Promega) contains two reporter genes for monitoring RNAi activity: a synthetic version of the renilla luciferase (hRluc) gene and a synthetic firefly luciferase gene (hluc+). Measurement of firefly luciferase activity permits determination of changes unrelated to the RNAi activity of tested dsRNA. Renilla and firefly luciferase activities were measured using the Dual-Glo® Luciferase Assay System (Promega). HBV target sites of interest were inserted into the psiCHECK™-2 vector, after cloning into the multiple cloning region located 3′ of the synthetic renilla luciferase gene's translational stop codon and the polyA tail . Cell line COS-7 was transfected with the vector, and subsequently treated with dsRNA-lipofectamine 2000 lipoplexes targeting the HBV sequences. The RNAi effect conferred by the dsRNA towards the cloned HBV target site was determined by measuring activity of the renilla luciferase fusion gene.

Generation of psiCHECK Vectors Containing Target Sequences. In order to test the activity of the HBV dsRNAs, a Dual-Luciferase HBV reporter was constructed. Regions 84 to 805, 1075 to 1992, 2165 to 2530, and 2718 to 2940 of Hepatitis B Virus genomic sequence accession number EU554538.1 (genotype C) were joined *in silico*. Two mutations were inserted intentionally (128 A→T, 598 T→C, positions relative to EU554538.1). One was needed to remove an internal XhoI site. The second mutation led to removal of a single mismatch to a dsRNA. This HBV target construct was extended by adding restriction sites at both the 5′ and 3′ end. The artificial DNA sequence was chemically synthesized by Geneart (Regensburg, Germany) and cloned into the XhoI

/NotI site of psiCHECK™-2 Dual-Luciferase vector.

Transfection and Luciferase Quantification. Cos-7 cells (DSMZ, Braunschweig, Germany, cat. No. ACC-60) were seeded at a density of 2.25×10^4 cells/well in 96-well plates. Plasmid transfection was carried out at a concentration of 50 ng/well with 0.5 µL/well Lipofectamine 2000 (Invitrogen GmbH, Karlsruhe, Germany, cat. No. 11668-019) as described by the manufacturer. 4 h after vector transfection, the medium was discarded and fresh medium was added. After this period, the dsRNAs were added to the cells in a concentration of 10 nM or 1 nM using Lipofectamine 2000 as described above. In order to optimize the HBV genotype coverage and to minimize development of resistance against dsRNAs, two different dsRNAs can be used simultaneously in combination. For demonstrating the feasibility of such approach, pairs of two different dsRNAs were selected among the most efficient dsRNAs with additional bias towards optimized genotype coverage.

The dsRNAs were added to the cells in a concentration of 5 nM or 0.5 nM for each dsRNA, resulting in 10 nM or 1 nM total dsRNA concentration, using Lipofectamine 2000 as described above. The cells were lysed 48 hours later using luciferase reagents as described by the manufacturer. Renilla luciferase protein levels were normalized to firefly luciferase levels to consider transfection efficiency. For each dsRNA four individual data points were collected. At least one dsRNA unrelated to all target sites was used as a control to determine the relative renilla luciferase protein levels in cells treated with dsRNA (Table 8). For comparison of silencing activity under full-match conditions, dsRNAs with full match to the renilla open reading frame were synthesized and tested in parallel to the HBV dsRNAs.

Inhibition data are given in appended Table 2.

Stability of dsRNAs. Stability of dsRNAs targeting human Hepatitis B Virus was determined in *in vitro* assays with any one of human, cynomolgous monkey or mouse serum by measuring the half-life of each single strand.

Measurements were carried out in triplicates for each time point, using 3 µL 50 µM dsRNA sample mixed with 30 µL human serum (Sigma), cynomolgous monkey serum

(Sigma) or mouse serum (Sigma). Mixtures were incubated for either 0 min, 30min, 1h, 3h, 6h, 24h, or 48h at 37°C. As control for unspecific degradation dsRNA was incubated with 30 µL 1× PBS pH 6.8 for 48h. Reactions were stopped by the addition of 4 µL proteinase K (20 mg/ml), 25 µL of"Tissue and Cell Lysis Solution" (Epicentre) and 38 µL Millipore water for 30 min at 65°C. Samples were afterwards spin filtered through a 0.2 µm 96 well filter plate at 1400 rpm for 8 min, washed with 55 µL Millipore water twice and spin filtered again.

For separation of single strands and analysis of remaining full length product (FLP), samples were run through an ion exchange Dionex Summit HPLC under denaturing conditions using as eluent A 20mM Na_3P0^4 in 10% ACN pH=11 and for eluent B 1M NaBr in eluent A.

The following gradient was applied:

Time (min)	%A	%B
−1.0	75	25
1.00	75	25
19.0	38	62
19.5	0	100
21.5	0	100
22.0	75	25
24.0	75	25

For every injection, the chromatograms were integrated automatically by the Dionex Chromeleon 6.60 HPLC software, and were adjusted manually if necessary. All peak areas were corrected to the internal standard (IS) peak and normalized to the incubation at t = 0 min. The area under the peak and resulting remaining FLP was calculated for each single strand and triplicate separately. Half-life (t½) of a strand was defined by the average time point (h) for triplicates at which half of the FLP was degraded. Results are given in appended Table 3.

CLAIMS:

1. A double-stranded ribonucleic acid molecule capable of inhibiting the expression of a Hepatitis B Virus gene wherein said double-stranded ribonucleic acid molecule comprises a sense strand comprising in order nucleotides 1-19 of SEQ ID NO: 2 and an antisense strand at least partially complementary to the sense strand and wherein said sense strand is less than 30 nucleotides in length.

2. The double-stranded ribonucleic acid molecule of claim 1, wherein said antisense strand comprises in order nucleotides 1-19 of SEQ ID NO: 158.

3. The double-stranded ribonucleic acid molecule of claim 1, wherein the antisense strand further comprises a 3' overhang of 1-5 nucleotides in length.

4. The double-stranded ribonucleic acid molecule of claim 3, wherein the 3' overhang of the antisense strand comprises uracil.

5. The double-stranded ribonucleic acid molecule of claim 1, wherein the sense strand further comprises a 3' overhang of 1-5 nucleotides in length.

6. The double-stranded ribonucleic acid molecule of claim 5 wherein the 3' overhang of the sense strand comprises uracil.

7. The double-stranded ribonucleic acid molecule of claim 1. wherein said double stranded ribonucleic acid molecule comprises at least one modified nucleotide which is: a 2'-0-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, a terminal nucleotide linked to a cholesteryl derivative, a terminal nucleotide linked to a dodecanoic acid bisdecylamide group, a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, a deoxythymidine, an inverted deoxythymidine, a 2'-amino-modified nucleotide, a 2'-

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alkyl-modified nucleotide, a morpholino nucleotide, a phosphoramidate, or a non-natural base comprising nucleotide.

8. The double-stranded ribonucleic acid molecule of claim 7, wherein said double stranded ribonucleic acid molecule contains a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a deoxythymidine.

9. The double-stranded ribonucleic acid molecule of claim 8, wherein said sense strand or said antisense strand comprises an overhang of 1-2 deoxythymidines.

10. The double-stranded ribonucleic acid molecule of claim 1, wherein said double-stranded ribonucleic acid molecule comprises the sequence pair of SEQ ID NOs: 322/486.

11. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent, and/or excipient, and:

a) at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10; or

b) a cell comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10.

12. A pharmaceutical composition comprising a pharmaceutically acceptable carrier, diluent and/or excipient, and:

a) a first and a second double-stranded ribonucleic acid molecules, wherein said first double-stranded ribonucleic acid molecule is as defined in any one of claims 1-10, and the second double-stranded ribonucleic acid molecule comprises a sense strand less than 30 nucleotides in length comprising, in order, nucleotides 1-19 of SEQ ID NO: 1-156 and an antisense strand at least partially complementary to the sense strand; or

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b) a cell comprising a first and a second double-stranded ribonucleic acid molecules, wherein said first double-stranded ribonucleic acid molecule is as defined in any one of claims 1-10, and the second double-stranded ribonucleic acid molecule comprises a sense strand less than 30 nucleotides in length comprising, in order, nucleotides 1-19 of SEQ ID NO: 1-156 and an antisense strand at least partially complementary to the sense strand.

13. The pharmaceutical composition of claim 12, wherein said second double-stranded ribonucleic acid comprises a sense strand comprising in order nucleotides 1-19 of SEQ ID NO: 3 or 6.

14. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus, comprising a pharmaceutically acceptable carrier, diluent, and/or excipient, and:

a) at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10;

b) at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10; or,

c) a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10.

15. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus, comprising a pharmaceutically acceptable carrier, diluent, and/or excipient, and:

a) a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10;

b) at least one nucleic acid sequence encoding a sense strands and an antisense strands of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the

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second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10; or,

c) a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10.

16. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, comprising a pharmaceutically acceptable carrier, diluent, and/or excipient, and:

a) at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10;

b) at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10; or,

c) a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10.

17. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, comprising a pharmaceutically acceptable carrier, diluent, and/or excipient, and:

a) a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10;

b) at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10; or,

c) a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10.

18. The double-stranded ribonucleic acid molecule of any one of claims 1-10, wherein the sense strand of SEQ ID NO: 2 is modified according to SEQ ID NO: 322.

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19. The double-stranded ribonucleic acid molecule of claim 1, wherein the antisense strand comprises in order nucleotides 2-18 of SEQ ID NO: 158.

20. The double-stranded ribonucleic acid molecule of claim 2 or 21, wherein the antisense strand of SEQ ID NO: 158 is modified according to SEQ ID NO: 486.

21. The double-stranded ribonucleic acid molecule of any one of claims 1-10 and 18-20, wherein the ribonucleic acid molecule is conjugated to a ligand.

22. The double-stranded ribonucleic acid molecule of claim 21, wherein the ligand is selected from the group consisting of galactose, folic acid, cholesterol, polyethylene glycols, carbohydrate clusters, cross-linking agents, porphyrin conjugates and delivery peptides.

23. The double-stranded ribonucleic acid molecule of claim 21, wherein the ligand comprises a galactose.

24. The double-stranded ribonucleic acid molecule as defined in any of claims 1-10 and 18-23, wherein the double-stranded ribonucleic acid molecule is present in a concentration in the range of 0.5-10 nM.

25. The double-stranded ribonucleic acid molecule of claim 24, wherein the double-stranded ribonucleic acid molecule is present in a concentration of 0.5 nM, 1 nM, 5 nM or 10 nM.

26. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

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27. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

28. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

29. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

30. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

31. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

32. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition

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comprises at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

33. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

34. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

35. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

36. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

37. A pharmaceutical composition for treating or preventing diseases caused by the infection of a Hepatitis B Virus comprising a cell, tissue or non-human organism comprising a

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first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

38. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

39. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising at least one nucleic acid sequence encoding a sense strands and an antisense strands of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

40. A pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus comprising a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

41. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

42. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense

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strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

43. Use of an effective amount of a pharmaceutical composition for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

44. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

45. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

46. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

47. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

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48. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

49. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

50. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

51. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

52. A pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene comprising a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

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53. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

54. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

55. Use of an effective amount of a pharmaceutical composition for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

56. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

57. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein

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the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

58. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

59. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

60. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

61. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for treating diseases caused by the infection of a Hepatitis B Virus, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as

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defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

62. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

63. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of the double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

64. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising at least one double-stranded ribonucleic acid molecule as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

65. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10.

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66. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises at least one nucleic acid sequence encoding a sense strand and an antisense strand of a first and a second double-stranded ribonucleic acid molecules, wherein the first and the second double-stranded ribonucleic acid molecules are each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

67. Use of an effective amount of a pharmaceutical composition in the preparation of a medicament for inhibiting the expression of a Hepatitis B virus gene, wherein the pharmaceutical composition comprises a cell, tissue or non-human organism comprising a first and a second double-stranded ribonucleic acid molecules each as defined in any one of claims 1-10, and a pharmaceutically acceptable carrier, diluent, and/or excipient.

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Table 1. Core sequences of dsRNAs targeting Hepatitis B Virus gene.

SEQ ID NO	sense strand sequence (5'-3')	SEQ ID NO	antisense strand sequence (5'-3')
1	CAAGGUAUGUUGCCCGUUU	157	AAACGGGCAACAUACCUUG
2	CUGUAGGCAUAAAUUGGUA	158	TACCAAUUUAUGCCUACAG
3	UCUGCGGCGUUUUAUCAUA	159	UAUGAUAAAACGCCGCAGA
3	UCUGCGGCGUUUUAUCAUA	160	TAUGAUAAAACGCCGCAGA
4	ACCUCUGCCUAAUCAUCUC	161	GAGAUGAUUAGGCAGAGGU
5	UUUACUAGUGCCAUUUGUA	162	TACAAAUGGCACUAGUAAA
6	ACCUCUGCCUAAUCAUCUA	163	TAGAUGAUUAGGCAGAGGU
7	CUGUAGGCAUAAAUUGGUC	164	GACCAAUUUAUGCCUACAG
8	UGUCUGCGGCGUUUUAUCA	165	UGAUAAAACGCCGCAGACA
8	UGUCUGCGGCGUUUUAUCA	166	TGAUAAAACGCCGCAGACA
9	UACUAGUGCCAUUUGUUCA	167	UGAACAAAUGGCACUAGUA
9	UACUAGUGCCAUUUGUUCA	168	TGAACAAAUGGCACUAGUA
10	CAACUUUUUCACCUCUGCA	169	TGCAGAGGUGAAAAAGUUG
11	CCAUUUGUUCAGUGGUUCG	170	CGAACCACUGAACAAAUGG
12	CCAAGUGUUUGCUGACGCA	171	UGCGUCAGCAAACACUUGG
12	CCAAGUGUUUGCUGACGCA	172	TGCGUCAGCAAACACUUGG
13	CCAUUUGUUCAGUGGUUCA	173	TGAACCACUGAACAAAUGG
14	UUUACUAGUGCCAUUUGUU	174	AACAAAUGGCACUAGUAAA
15	CACCUCUGCCUAAUCAUCA	175	TGAUGAUUAGGCAGAGGUG
16	CUGGCUCAGUUUACUAGUG	176	CACUAGUAAACUGAGCCAG
17	CAAGGUAUGUUGCCCGUUA	177	TAACGGGCAACAUACCUUG
18	CUGGCUCAGUUUACUAGUA	178	TACUAGUAAACUGAGCCAG
19	GAGGCUGUAGGCAUAAAUU	179	AAUUUAUGCCUACAGCCUC
20	CAGUUUACUAGUGCCAUUU	180	AAAUGGCACUAGUAAACUG
21	AGGUAUGUUGCCCGUUUGU	181	ACAAACGGGCAACAUACCU
22	UAUGUUGCCCGUUUGUCCA	182	UGGACAAACGGGCAACAUA
23	GAGGCUGUAGGCAUAAAUA	183	TAUUUAUGCCUACAGCCUC
24	GUCUGCGGCGUUUUAUCAU	184	AUGAUAAAACGCCGCAGAC
25	CAACUUUUUCACCUCUGCC	185	GGCAGAGGUGAAAAAGUUG
26	CCGUGUGCACUUCGCUUCA	186	UGAAGCGAAGUGCACACGG
26	CCGUGUGCACUUCGCUUCA	187	TGAAGCGAAGUGCACACGG
27	UCAAGGUAUGUUGCCCGUA	188	TACGGGCAACAUACCUUGA
28	CAGUUUACUAGUGCCAUUA	189	TAAUGGCACUAGUAAACUG
29	UGGUGGACUUCUCUCAAUU	190	AAUUGAGAGAAGUCCACCA
30	AGGUAUGUUGCCCGUUUGA	191	TCAAACGGGCAACAUACCU
31	CUGCUCGUGUUACAGGCGG	192	CCGCCUGUAACACGAGCAG
32	UAUGUUGCCCGUUUGUCCU	193	AGGACAAACGGGCAACAUA
33	UCAAGGUAUGUUGCCCGUU	194	AACGGGCAACAUACCUUGA
34	UCUUAUCAACACUUCCGGA	195	UCCGGAAGUGUUGAUAAGA
34	UCUUAUCAACACUUCCGGA	196	TCCGGAAGUGUUGAUAAGA
35	CACCUCUGCCUAAUCAUCU	197	AGAUGAUUAGGCAGAGGUG
36	AUAAGAGGACUCUUGGACU	198	AGUCCAAGAGUCCUCUUAU
37	GUCUGCGGCGUUUUAUCAA	199	TUGAUAAAACGCCGCAGAC
38	GGCGCUGAAUCCCGCGGAC	200	GUCCGCGGGAUUCAGCGCC

FIG. 1

39	CGCGUCGCAGAAGAUCUCA	201	UGAGAUCUUCUGCGACGCG
40	AAUGUCAACGACCGACCUU	202	AAGGUCGGUCGUUGACAUU
41	GCUCAGUUUACUAGUGCCA	203	UGGCACUAGUAAACUGAGC
42	UGGUGGACUUCUCUCAAUA	204	TAUUGAGAGAAGUCCACCA
43	AUCGCCGCGUCGCAGAAGA	205	UCUUCUGCGACGCGGCGAU
44	GCCAUUUGUUCAGUGGUUC	206	GAACCACUGAACAAAUGGC
45	CGAUCCAUACUGCGGAACU	207	AGUUCCGCAGUAUGGAUCG
46	UCACCUCUGCCUAAUCAUC	208	GAUGAUUAGGCAGAGGUGA
47	GUGGACUUCUCUCAAUUUU	209	AAAAUUGAGAGAAGUCCAC
48	GGGUCACCAUAUUCUUGGG	210	CCCAAGAAUAUGGUGACCC
49	GCCGCGUCGCAGAAGAUCU	211	AGAUCUUCUGCGACGCGGC
50	UCAAUCGCCGCGUCGCAGA	212	UCUGCGACGCGGCGAUUGA
51	UGGAUGUGUCUGCGGCGUU	213	AACGCCGCAGACACAUCCA
52	UACUGUUCAAGCCUCCAAG	214	CUUGGAGGCUUGAACAGUA
53	GUUUACUAGUGCCAUUUGU	215	ACAAAUGGCACUAGUAAAC
54	ACUAGUGCCAUUUGUUCAG	216	CUGAACAAAUGGCACUAGU
55	CCGCGUCGCAGAAGAUCUC	217	GAGAUCUUCUGCGACGCGG
56	UAUCUUAUCAACACUUCCG	218	CGGAAGUGUUGAUAAGAUA
57	GGCCAAAAUUCGCAGUCCC	219	GGGACUGCGAAUUUUGGCC
58	UUCACCUCUGCCUAAUCAU	220	AUGAUUAGGCAGAGGUGAA
59	CUCAGUUUACUAGUGCCAU	221	AUGGCACUAGUAAACUGAG
60	UGUUGCCCGUUUGUCCUCU	222	AGAGGACAAACGGGCAACA
61	UAGUGCCAUUUGUUCAGUG	223	CACUGAACAAAUGGCACUA
62	AGGCUGUAGGCAUAAAUUG	224	CAAUUUAUGCCUACAGCCU
63	AUGUGUCUGCGGCGUUUUA	225	UAAAACGCCGCAGACACAU
63	AUGUGUCUGCGGCGUUUUA	226	TAAAACGCCGCAGACACAU
64	ACUUCGCUUCACCUCUGCA	227	UGCAGAGGUGAAGCGAAGU
65	CGUGUGCACUUCGCUUCAC	228	GUGAAGCGAAGUGCACACG
66	GUGGUGGACUUCUCUCAAU	229	AUUGAGAGAAGUCCACCAC
67	UGUGUCUGCGGCGUUUUAU	230	AUAAAACGCCGCAGACACA
68	AAGGUAUGUUGCCCGUUUG	231	CAAACGGGCAACAUACCUU
69	UCAACGACCGACCUUGAGG	232	CCUCAAGGUCGGUCGUUGA
70	CAUAAGAGGACUCUUGGAC	233	GUCCAAGAGUCCUCUUAUG
71	GUCAACGACCGACCUUGAG	234	CUCAAGGUCGGUCGUUGAC
72	AUAUUCUUGGGAACAAGAG	235	CUCUUGUUCCCAAGAAUAU
73	UGCUCGUGUUACAGGCGGG	236	CCCGCCUGUAACACGAGCA
74	CAAUCGCCGCGUCGCAGAA	237	UUCUGCGACGCGGCGAUUG
75	ACUGUUCAAGCCUCCAAGC	238	GCUUGGAGGCUUGAACAGU
76	CGCCGCGUCGCAGAAGAUC	239	GAUCUUCUGCGACGCGGCG
77	CAUUUGUUCAGUGGUUCGU	240	ACGAACCACUGAACAAAUG
78	CGCUGAAUCCCGCGGACGA	241	UCGUCCGCGGGAUUCAGCG
79	UGGGUCACCAUAUUCUUGG	242	CCAAGAAUAUGGUGACCCA
80	UCCUCUGCCGAUCCAUACU	243	AGUAUGGAUCGGCAGAGGA
81	AUGUCAACGACCGACCUUG	244	CAAGGUCGGUCGUUGACAU
82	CCUCUGCCUAAUCAUCUCA	245	UGAGAUGAUUAGGCAGAGG
83	ACCGUGUGCACUUCGCUUC	246	GAAGCGAAGUGCACACGGU
84	UGCCGAUCCAUACUGCGGA	247	UCCGCAGUAUGGAUCGGCA

FIG. 1

85	CAGAGUCUAGACUCGUGGU	248	ACCACGAGUCUAGACUCUG
86	CUGUUCAAGCCUCCAAGCU	249	AGCUUGGAGGCUUGAACAG
87	GGAGGCUGUAGGCAUAAAU	250	AUUUAUGCCUACAGCCUCC
88	AGGAGGCUGUAGGCAUAAA	251	UUUAUGCCUACAGCCUCCU
89	GGUGGACUUCUCUCAAUUU	252	AAAUUGAGAGAAGUCCACC
90	GCAACUUUUUCACCUCUGC	253	GCAGAGGUGAAAAAGUUGC
91	CUGCUCGUGUUACAGGCGA	254	TCGCCUGUAACACGAGCAG
92	CUAGUGCCAUUUGUUCAGU	255	ACUGAACAAAUGGCACUAG
93	CUGCCGAUCCAUACUGCGG	256	CCGCAGUAUGGAUCGGCAG
94	GUGUGCACUUCGCUUCACC	257	GGUGAAGCGAAGUGCACAC
95	GCUCGUGUUACAGGCGGGC	258	GCCCGCCUGUAACACGAGC
96	CCUAUCUUAUCAACACUUC	259	GAAGUGUUGAUAAGAUAGG
97	UCUCAAUCGCCGCGUCGCA	260	UGCGACGCGGCGAUUGAGA
98	GCCCGUCUGUGCCUUCUCA	261	UGAGAAGGCACAGACGGGC
99	CUAUCUUAUCAACACUUCC	262	GGAAGUGUUGAUAAGAUAG
100	AUGUUGCCCGUUUGUCCUC	263	GAGGACAAACGGGCAACAU
101	GUAUGUUGCCCGUUUGUCC	264	GGACAAACGGGCAACAUAC
102	CUUCGCUUCACCUCUGCAC	265	GUGCAGAGGUGAAGCGAAG
103	UGUGCACUUCGCUUCACCU	266	AGGUGAAGCGAAGUGCACA
104	GCCAAAAUUCGCAGUCCCG	267	CGGGACUGCGAAUUUUGGC
105	CCUGCUCGUGUUACAGGCG	268	CGCCUGUAACACGAGCAGG
106	UGGAGUGUGGAUUCGCACU	269	AGUGCGAAUCCACACUCCA
107	AACGACCGACCUUGAGGCA	270	UGCCUCAAGGUCGGUCGUU
108	ACAGAGUCUAGACUCGUGG	271	CCACGAGUCUAGACUCUGU
109	AAUCGCCGCGUCGCAGAAG	272	CUUCUGCGACGCGGCGAUU
110	GGUAUGUUGCCCGUUUGUC	273	GACAAACGGGCAACAUACC
111	GCCGAUCCAUACUGCGGAA	274	UUCCGCAGUAUGGAUCGGC
112	GCCCUAUCUUAUCAACACU	275	AGUGUUGAUAAGAUAGGGC
113	AGUUUACUAGUGCCAUUUG	276	CAAAUGGCACUAGUAAACU
114	UGUCAACGACCGACCUUGA	277	UCAAGGUCGGUCGUUGACA
115	ACUUCUCUCAAUUUUCUAG	278	CUAGAAAAUUGAGAGAAGU
116	GCGCGGGACGUCCUUUGUC	279	GACAAAGGACGUCCCGCGC
117	UCUAGACUCGUGGUGGACU	280	AGUCCACCACGAGUCUAGA
118	GAUCCAUACUGCGGAACUC	281	GAGUUCCGCAGUAUGGAUC
119	CUCUGCCGAUCCAUACUGC	282	GCAGUAUGGAUCGGCAGAG
120	UCUGCCGAUCCAUACUGCG	283	CGCAGUAUGGAUCGGCAGA
121	CCUCUGCCGAUCCAUACUG	284	CAGUAUGGAUCGGCAGAGG
122	GCACCUCUCUUUACGCGGU	285	ACCGCGUAAAGAGAGGUGC
123	AAGAACUCCCUCGCCUCGC	286	GCGAGGCGAGGGAGUUCUU
124	GAACUCCCUCGCCUCGCAG	287	CUGCGAGGCGAGGGAGUUC
125	UCUCUCAAUUUUCUAGGGC	288	GCCCUAGAAAAUUGAGAGA
126	GGGCGCACCUCUCUUUACG	289	CGUAAAGAGAGGUGCGCCC
127	CCGAUCCAUACUGCGGAAC	290	GUUCCGCAGUAUGGAUCGG
128	AACUCCCUCGCCUCGCAGA	291	UCUGCGAGGCGAGGGAGUU
129	CUCCUCUGCCGAUCCAUAC	292	GUAUGGAUCGGCAGAGGAG
130	GGAGUGUGGAUUCGCACUC	293	GAGUGCGAAUCCACACUCC
131	CGGGCGCACCUCUCUUUAC	294	GUAAAGAGAGGUGCGCCCG

FIG. 1

132	GUCUCAAUCGCCGCGUCGC	295	GCGACGCGGCGAUUGAGAC
133	AUCCAUACUGCGGAACUCC	296	GGAGUUCCGCAGUAUGGAU
134	CGCACCUCUCUUUACGCGG	297	CCGCGUAAAGAGAGGUGCG
135	CAACGACCGACCUUGAGGC	298	GCCUCAAGGUCGGUCGUUG
136	CCAUACUGCGGAACUCCUA	299	UAGGAGUUCCGCAGUAUGG
137	UGAAUCCCGCGGACGACCC	300	GGGUCGUCCGCGGGAUUCA
138	AGAACUCCCUCGCCUCGCA	301	UGCGAGGCGAGGGAGUUCU
139	GGCGCACCUCUCUUUACGC	302	GCGUAAAGAGAGGUGCGCC
140	GCGCACCUCUCUUUACGCG	303	CGCGUAAAGAGAGGUGCGC
141	GCUGAAUCCCGCGGACGAC	304	GUCGUCCGCGGGAUUCAGC
142	CACUUCGCUUCACCUCUGC	305	GCAGAGGUGAAGCGAAGUG
143	CUCAAUCGCCGCGUCGCAG	306	CUGCGACGCGGCGAUUGAG
144	UCCCGUCGGCGCUGAAUCC	307	GGAUUCAGCGCCGACGGGA
145	CUGAAUCCCGCGGACGACC	308	GGUCGUCCGCGGGAUUCAG
146	AGAGUCUAGACUCGUGGUG	309	CACCACGAGUCUAGACUCU
147	UCCAUACUGCGGAACUCCU	310	AGGAGUUCCGCAGUAUGGA
148	GCGCUGAAUCCCGCGGACG	311	CGUCCGCGGGAUUCAGCGC
149	AGUGUGGAUUCGCACUCCU	312	AGGAGUGCGAAUCCACACU
150	CCCUGCUCGUGUUACAGGC	313	GCCUGUAACACGAGCAGGG
151	GAAUCCCGCGGACGACCCG	314	CGGGUCGUCCGCGGGAUUC
152	AAGCUGUGCCUUGGGUGGC	315	GCCACCCAAGGCACAGCUU
153	GCCCUGCUCGUGUUACAGG	316	CCUGUAACACGAGCAGGGC
154	GUCCCGUCGGCGCUGAAUC	317	GAUUCAGCGCCGACGGGAC
155	AUCUUAUCAACACUUCCGG	318	CCGGAAGUGUUGAUAAGAU
156	CUUAUCAACACUUCCGGAA	319	UUCCGGAAGUGUUGAUAAG
156	CUUAUCAACACUUCCGGAA	320	TUCCGGAAGUGUUGAUAAG

FIG. 1

Table 2. Activity testing in psiCHECK2 reporter system in COS7 cells.

SEQ ID NO	sense strand sequence (5'-3')	SEQ ID NO	antisense strand sequence (5'-3')	Activity testing in psiCHECK2 reporter system in COS7 cells			
				10 nM siRNA		**1 nM siRNA**	
				mean remaining mRNA (%)	standard deviation (%)	mean remaining mRNA (%)	standard deviation (%)
321	caAGGuAuGuuGcccGuuudTsdT	485	AAACGGGcAAcAuACCUUGdTsdT	13	1	13	1
322	CfuGfuAfgGfcAfuAfaAfuUfgGfuAf(invdT)	486	pdTAfcCfaAfuUfuAfuGfcCfuAfcAfgdTsdT	8	2	14	2
323	ucuGcGGcGuuuuAucAuAdTsdT	487	uAUGAuAAAACGCCGcAGAdTsdT	15	7	29	11
324	UfcUfgCfgGfcGfuUfuUfaUfcAfuAf(invdT)	488	pdTAfuGfaUfaAfaAfcGfcCfgCfaGfadTsdT	6	2	15	4
325	accucuGccuAAucAucucdTsdT	489	GAGAUGAUuAGGcAGAGGUdTsdT	17	1	16	2
326	UfuUfaCfuAfgUfgCfcAfuUfuGfuAf(invdT)	490	pdTAfcAfaAfuGfgCfaCfuAfgUfaAfadTsdT	8	0	17	1
327	AfcCfuCfuGfcCfuAfaUfcAfuCfuAf(invdT)	491	pdTAfgAfuGfaUfuAfgGfcAfgAfgGfudTsdT	6	2	19	3
328	cuGuAGGcAuAAAuuGGucdTsdT	492	GACcAAUUuAUGCCuAcAGdTsdT	23	2	28	6
329	ugucuGcGGcGuuuuAucAdTsdT	493	UGAuAAAACGCCGcAGAcAdTsdT	33	3	34	10
330	UfgUfcUfgCfgGfcGfuUfuUfaUfcAf(invdT)	494	pdTGfaUfaAfaAfcGfcCfgCfaGfaCfadTsdT	6	0	20	3
331	uacuAGuGccAuuuGuucAdTsdT	495	UGAAcAAAUGGcACuAGuAdTsdT	18	3	20	2
332	UfaCfuAfgUfgCfcAfuUfuGfuUfcAf(invdT)	496	pdTGfaAfcAfaAfuGfgCfaCfuAfgUfadTsdT	6	2	21	4
333	CfaAfcUfuUfuUfcAfcCfuCfuGfcAf(invdT)	497	pdTGfcAfgAfgGfuGfaAfaAfaGfuUfgdTsdT	6	2	21	3
334	ccAuuuGuucAGuGGuucGdTsdT	498	CGAACcACUGAAcAAAUGGdTsdT	12	1	21	1
335	ccAAGuGuuuGcuGAcGcAdTsdT	499	UGCGUcAGcAAAcACUUGGdTsdT	18	2	23	4
336	CfcAfaGfuGfuUfuGfcUfgAfcGfcAf(invdT)	500	pdTGfcGfuCfaGfcAfaAfcAfcUfuGfgdTsdT	8	1	23	5
337	CfcAfuUfuGfuUfcAfgUfgGfuUfcAf(invdT)	501	pdTGfaAfcCfaCfuGfaAfcAfaAfuGfgdTsdT	7	2	24	3
338	uuuAcuAGuGccAuuuGuudTsdT	502	AAcAAAUGGcACuAGuAAAdTsdT	21	2	24	4
339	CfaCfcUfcUfgCfcUfaAfuCfaUfcAf(invdT)	503	pdTGfaUfgAfuUfaGfgCfaGfaGfgUfgdTsdT	9	0	25	2
340	cuGGcucAGuuuAcuAGuGdTsdT	504	cACuAGuAAACUGAGCcAGdTsdT	34	3	29	7
341	CfaAfgGfuAfuGfuUfgCfcCfgUfuAf(invdT)	505	pdTAfaCfgGfgCfaAfcAfuAfcCfuUfgdTsdT	8	0	31	3
342	CfuGfgCfuCfaGfuUfuAfcUfaGfuAf(invdT)	506	pdTAfcUfaGfuAfaAfcUfgAfgCfcAfgdTsdT	11	3	32	5
343	gaGGcuGuAGGcAuAAAuudTsdT	507	AAUUuAUGCCuAcAGCCUCdTsdT	16	1	32	8

FIG. 2

344	caGuuuAcuAGuGccAuuudTsdT	508	AAAUGGcACuAGuAAACUGdTsdT	37	1	33	8
345	agGuAuGuuGcccGuuuGudTsdT	509	AcAAACGGGcAAcAuACCUdTsdT	33	3	34	4
346	UfaUfgUfuGfcCfcGfuUfuGfuCfcAf(invdT)	510	pdTGfgAfcAfaAfcGfgGfcAfaCfaUfadTsdT	9	3	35	5
347	GfaGfgCfuGfuAfgGfcAfuAfaAfuAf(invdT)	511	pdTAfuUfuAfuGfcCfuAfcAfgCfcUfcdTsdT	9	1	36	4
348	gucuGcGGcGuuuuAucAudTsdT	512	AUGAuAAAACGCCGcAGACdTsdT	26	3	36	14
349	caAcuuuuucAccucuGccdTsdT	513	GGcAGAGGUGAAAAAGUUGdTsdT	24	2	37	9
350	ccGuGuGcAcuucGcuucAdTsdT	514	UGAAGCGAAGUGcAcACGGdTsdT	13	1	16	5
351	CfcGfuGfuGfcAfcUfuCfgCfuUfcAf(invdT)	515	pdTGfaAfgCfgAfaGfuGfcAfcAfcGfgdTsdT	13	2	38	4
352	UfcAfaGfgUfaUfgUfuGfcCfcGfuAf(invdT)	516	pdTAfcGfgGfcAfaCfaUfaCfcUfuGfadTsdT	12	1	38	4
353	CfaGfuUfuAfcUfaGfuGfcCfaUfuAf(invdT)	517	pdTAfaUfgGfcAfcUfaGfuAfaAfcUfgdTsdT	12	2	38	5
354	ugGuGGAcuucucucAAuudTsdT	518	AAUUGAGAGAAGUCcACcAdTsdT	24	6	39	16
355	AfgGfuAfuGfuUfgCfcCfgUfuUfgAf(invdT)	519	pdTCfaAfaCfgGfgCfaAfcAfuAfcCfudTsdT	18	1	40	4
356	cuGcucGuGuuAcAGGcGGdTsdT	520	CCGCCUGuAAcACGAGcAGdTsdT	26	2	40	11
357	uauGuuGcccGuuuGuccudTsdT	521	AGGAcAAACGGGcAAcAuAdTsdT	42	1	40	3
358	ucAAGGuAuGuuGcccGuudTsdT	522	AACGGGcAAcAuACCUUGAdTsdT	31	4	42	12
359	ucuuAucAAcAcAcuuccGGAdTsdT	523	UCCGGAAGUGUUGAuAAGAdTsdT	35	2	43	38
360	UfcUfuAfuCfaAfcAfcUfuCfcGfgAf(invdT)	524	pdTCfcGfgAfaGfuGfuUfgAfuAfaGfadTsdT	32	2	46	3
361	caccucuGccuAAucAucudTsdT	525	AGAUGAUuAGGcAGAGGUGdTsdT	31	3	47	8
362	auAAGAGGAcucuuGGAcudTsdT	526	AGUCcAAGAGUCCUCUuAUdTsdT	28	1	49	6
363	GfuCfuGfcGfgCfgUfuUfuAfuCfaAf(invdT)	527	pdTUfgAfuAfaAfaCfgCfcGfcAfgAfcdTsdT	15	0	51	4
364	ggcGcuGAAucccGcGGAcdTsdT	528	GUCCGCGGGAUUcAGCGCCdTsdT	24	3	51	10
365	cgcGucGcAGAAGAucucAdTsdT	529	UGAGAUCUUCUGCGACGCGdTsdT	46	3	53	6
366	aauGucAAcGAccGAccuudTsdT	530	AAGGUCGGUCGUUGAcAUUdTsdT	40	1	54	8
367	gcucAGuuuAcuAGuGccAdTsdT	531	UGGcACuAGuAAACUGAGCdTsdT	37	5	51	4
368	UfgGfuGfgAfcUfuCfuCfuCfaAfuAf(invdT)	532	pdTAfuUfgAfgAfgAfaGfuCfcAfcCfadTsdT	20	4	58	6
369	aucGccGcGucGcAGAAGAdTsdT	533	UCUUCUGCGACGCGGCGAUdTsdT	57	6	58	1
370	gccAuuuGuucAGuGGuucdTsdT	534	GAACcACUGAAcAAAUGGCdTsdT	36	3	60	6
371	cgAuccAuAcuGcGGAAcudTsdT	535	AGUUCCGcAGuAUGGAUCGdTsdT	43	8	61	9
372	ucAccucuGccuAAucAucdTsdT	536	GAUGAUuAGGcAGAGGUGAdTsdT	48	4	61	10
373	guGGAcuucucucAAuuudTsdT	537	AAAAUUGAGAGAAGUCcACdTsdT	31	4	61	5
374	ggGucAccAuAuucuuGGGdTsdT	538	CCcAAGAAuAUGGUGACCCdTsdT	58	6	62	10

FIG. 2

375	gccGcGucGcAGAAGAucudTsdT	539	AGAUCUUCUGCGACGCGGCdTsdT	59	3	64	7
376	ucAAucGccGcGucGcAGAdTsdT	540	UCUGCGACGCGGCGAUUGAdTsdT	59	1	64	9
377	ugGAuGuGucuGcGGcGuudTsdT	541	AACGCCGcAGAcAcAUCcAdTsdT	44	8	65	12
378	uacuGuucAAGccuccAAGdTsdT	542	CUUGGAGGCUUGAAcAGuAdTsdT	51	2	65	32
379	guuuAcuAGuGccAuuuGudTsdT	543	AcAAAUGGcACuAGuAAACdTsdT	44	5	66	6
380	acuAGuGccAuuuGuucAGdTsdT	544	CUGAAcAAAUGGcACuAGUdTsdT	56	0	66	5
381	ccGcGucGcAGAAGAucucdTsdT	545	GAGAUCUUCUGCGACGCGGdTsdT	59	3	67	11
382	uaucuuAucAAcAcuuccGdTsdT	546	CGGAAGUGUUGAuAAGAuAdTsdT	37	1	67	51
383	ggccAAAAuucGcAGucccdTsdT	547	GGGACUGCGAAUUUUGGCCdTsdT	49	6	67	7
384	uucAccucuGccuAAucAudTsdT	548	AUGAUuAGGcAGAGGUGAAdTsdT	50	4	68	7
385	cucAGuuuAcuAGuGccAudTsdT	549	AUGGcACuAGuAAACUGAGdTsdT	52	2	68	6
386	uguuGcccGuuuGuccucudTsdT	550	AGAGGAcAAACGGGcAAcAdTsdT	50	2	69	4
387	uaGuGccAuuuGuucAGuGdTsdT	551	cACUGAAcAAAUGGcACuAdTsdT	46	1	70	8
388	agGcuGuAGGcAuAAAuuGdTsdT	552	cAAUUuAUGCCuAcAGCCUdTsdT	69	3	71	13
389	auGuGucuGcGGcGuuuuAdTsdT	553	uAAAACGCCGcAGAcAcAUdTsdT	17	6	33	12
390	AfuGfuGfuCfuGfcGfgCfgUfuUfuAf(invdT)	554	pdTAfaAfaCfgCfcGfcAfgAfcAfcAfudTsdT	24	3	72	4
391	acuucGcuucAccucuGcAdTsdT	555	UGcAGAGGUGAAGCGAAGUdTsdT	49	4	73	4
392	cguGuGcAcuucGcuucAcdTsdT	556	GUGAAGCGAAGUGcAcACGdTsdT	45	3	73	10
393	guGGuGGAcuucucucAAudTsdT	557	AUUGAGAGAAGUCcACcACdTsdT	47	5	73	5
394	uguGucuGcGGcGuuuuAudTsdT	558	AuAAAACGCCGcAGAcAcAdTsdT	62	8	75	14
395	aaGGuAuGuuGcccGuuuGdTsdT	559	cAAACGGGcAAcAuACCUUdTsdT	61	3	76	2
396	ucAAcGAccGAccuuGAGGdTsdT	560	CCUcAAGGUCGGUCGUUGAdTsdT	57	1	76	16
397	cauAAGAGGAcucuuGGAcdTsdT	561	GUCcAAGAGUCCUCUuAUGdTsdT	62	4	76	4
398	gucAAcGAccGAccuuGAGdTsdT	562	CUcAAGGUCGGUCGUUGACdTsdT	55	2	77	13
399	auAuucuuGGGAAcAAGAGdTsdT	563	CUCUUGUUCCcAAGAAuAUdTsdT	56	5	77	11
400	ugcucGuGuuAcAGGcGGGdTsdT	564	CCCGCCUGuAAcACGAGcAdTsdT	61	5	78	4
401	caAucGccGcGucGcAGAAdTsdT	565	UUCUGCGACGCGGCGAUUGdTsdT	65	1	78	4
402	acuGuucAAGccuccAAGcdTsdT	566	GCUUGGAGGCUUGAAcAGUdTsdT	86	3	78	5
403	cgccGcGucGcAGAAGAucdTsdT	567	GAUCUUCUGCGACGCGGCGdTsdT	71	3	79	2
404	cauuuGuucAGuGGuucGudTsdT	568	ACGAACcACUGAAcAAAUGdTsdT	54	5	80	4
405	cgcuGAAucccGcGGAcGAdTsdT	569	UCGUCCGCGGGAUUcAGCGdTsdT	62	2	80	7

FIG. 2

406	ugGGucAccAuAuucuuGGdTsdT	570	CcAAGAAuAUGGUGACCcAdTsdT	75	2	80	16
407	uccucuGccGAuccAuAcudTsdT	571	AGuAUGGAUCGGcAGAGGAdTsdT	73	2	81	3
408	auGucAAcGAccGAccuuGdTsdT	572	cAAGGUCGGUCGUUGAcAUdTsdT	69	8	81	7
409	ccucuGccuAAucAucucAdTsdT	573	UGAGAUGAUuAGGcAGAGGdTsdT	81	4	81	4
410	accGuGuGcAcuucGcuucdTsdT	574	GAAGCGAAGUGcAcACGGUdTsdT	46	5	81	7
411	ugccGAuccAuAcuGcGGAdTsdT	575	UCCGcAGuAUGGAUCGGcAdTsdT	61	8	81	5
412	caGAGucuAGAcucGuGGudTsdT	576	ACcACGAGUCuAGACUCUGdTsdT	65	9	81	5
413	cuGuucAAGccuccAAGcudTsdT	577	AGCUUGGAGGCUUGAAcAGdTsdT	82	3	82	21
414	ggAGGcuGuAGGcAuAAAudTsdT	578	AUUuAUGCCuAcAGCCUCCdTsdT	68	2	82	12
415	agGAGGcuGuAGGcAuAAAdTsdT	579	UUuAUGCCuAcAGCCUCCUdTsdT	55	4	83	5
416	gguGGAcuucucucAAuuudTsdT	580	AAAUUGAGAGAAGUCcACCdTsdT	62	7	84	2
417	gcAAcuuuuuucAccucuGcdTsdT	581	GcAGAGGUGAAAAAAGUUGCdTsdT	93	1	85	5
418	CfuGfcUfcGfuGfuUfaCfaGfgCfgAf(invdT)	582	pdTCfgCfcUfgUfaAfcAfcGfaGfcAfgdTsdT	56	1	86	2
419	cuAGuGccAuuuGuucAGudTsdT	583	ACUGAAcAAAUGGcACuAGdTsdT	66	0	86	6
420	cuGccGAuccAuAcuGcGGdTsdT	584	CCGcAGuAUGGAUCGGcAGdTsdT	73	8	86	5
421	guGuGcAcuucGcuucAccdTsdT	585	GGUGAAGCGAAGUGcAcACdTsdT	54	4	87	4
422	gcucGuGuuAcAGGcGGGcdTsdT	586	GCCCGCCUGuAAcACGAGCdTsdT	91	4	87	5
423	ccuAucuuAucAAcAcuucdTsdT	587	GAAGUGUUGAuAAGAuAGGdTsdT	37	2	88	45
424	ucucAAucGccGcGucGcAdTsdT	588	UGCGACGCGGCGAUUGAGAdTsdT	79	4	88	6
425	gcccGucuGuGcccuucucAdTsdT	589	UGAGAAGGcAcAGACGGGCdTsdT	85	4	88	16
426	cuAucuuAucAAcAcuucccdTsdT	590	GGAAGUGUUGAuAAGAuAGdTsdT	43	3	90	23
427	auGuuGcccGuuuGucccucdTsdT	591	GAGGAcAAACGGGcAAcAUdTsdT	87	5	90	4
428	guAuGuuGcccGuuuGucccdTsdT	592	GGAcAAACGGGcAAcAuACdTsdT	88	4	90	11
429	cuucGcuucAccucuGcAccdTsdT	593	GUGcAGAGGUGAAGCGAAGdTsdT	69	7	91	5
430	uguGcAcuucGcuucAccudTsdT	594	AGGUGAAGCGAAGUGcAcAdTsdT	76	3	91	14
431	gccAAAAuucGcAGucccGdTsdT	595	CGGGACUGCGAAUUUUGGCdTsdT	81	3	92	3
432	ccuGcuGuGuuAcAGGcGdTsdT	596	CGCCUGuAAcACGAGcAGGdTsdT	86	3	92	1
433	ugGAGuGuGGAuucGcAcudTsdT	597	AGUGCGAAUCcAcACUCcAdTsdT	87	4	92	3
434	aacGAccGAccuuGAGGcAdTsdT	598	UGCCUcAAGGUCGGUCGUUdTsdT	83	9	92	3
435	acAGAGucuAGAcucGuGGdTsdT	599	CcACGAGUCuAGACUCUGUdTsdT	89	4	92	4
436	aaucGccGcGucGcAGAAGdTsdT	600	CUUCUGCGACGCGGCGAUUdTsdT	85	6	92	2

FIG. 2

437	gguAuGuuGcccGuuuGucdTsdT	601	GAcAAACGGGcAAcAuACCdTsdT	80	2	93	3
438	gccGAuccAuAcuGcGGAAdTsdT	602	UUCCGcAGuAUGGAUCGGCdTsdT	79	3	93	3
439	gcccuAucuuAucAAcAcudTsdT	603	AGUGUUGAuAAGAuAGGGCdTsdT	84	4	94	50
440	aguuuAcuAGuGccAuuuGdTsdT	604	cAAAUGGcACuAGuAAACUdTsdT	89	7	95	8
441	ugucAAcGAccGAccuuGAdTsdT	605	UcAAGGUCGGUCGUUGAcAdTsdT	84	5	95	8
442	acuucucucAAuuuuucuAGdTsdT	606	CuAGAAAAUUGAGAGAAGUdTsdT	103	3	95	6
443	gcGcGGGAcGuccuuuGucdTsdT	607	GAcAAAGGACGUCCCGCGCdTsdT	88	4	97	3
444	ucuAGAcucGuGGuGGAcudTsdT	608	AGUCcACcACGAGUCuAGAdTsdT	90	5	97	2
445	gauccAuAcuGcGGAAcucdTsdT	609	GAGUUCCGcAGuAUGGAUCdTsdT	73	6	98	4
446	cucuGccGAuccAuAcuGcdTsdT	610	GcAGuAUGGAUCGGcAGAGdTsdT	100	5	99	7
447	ucuGccGAuccAuAcuGcGdTsdT	611	CGcAGuAUGGAUCGGcAGAdTsdT	88	6	99	4
448	ccucuGccGAuccAuAcuGdTsdT	612	cAGuAUGGAUCGGcAGAGGdTsdT	98	11	99	5
449	gcAccucucuuuAcGcGGudTsdT	613	ACCGCGuAAAGAGAGGUGCdTsdT	82	7	100	4
450	aaGAAcucccucGccucGcdTsdT	614	GCGAGGCGAGGGAGUUCUUdTsdT	97	6	100	1
451	gaAcucccucGccucGcAGdTsdT	615	CUGCGAGGCGAGGGAGUUCdTsdT	100	2	100	2
452	ucucucAAuuuuucuAGGGcdTsdT	616	GCCCuAGAAAAUUGAGAGAdTsdT	102	4	100	8
453	ggGcGcAccucucuuuAcGdTsdT	617	CGuAAAGAGAGGUGCGCCCdTsdT	80	4	100	3
454	ccGAuccAuAcuGcGGAAccdTsdT	618	GUUCCGcAGuAUGGAUCGGdTsdT	83	5	101	3
455	aacucccucGccucGcAGAdTsdT	619	UCUGCGAGGCGAGGGAGUUdTsdT	100	2	101	2
456	cuccucuGccGAuccAuAccdTsdT	620	GuAUGGAUCGGcAGAGGAGdTsdT	93	2	101	2
457	ggAGuGuGGAuucGcAcucdTsdT	621	GAGUGCGAAUCcAcACUCCdTsdT	97	5	101	3
458	cgGGcGcAccucucuuuAcdTsdT	622	GuAAAGAGAGGUGCGCCCGdTsdT	83	6	101	6
459	gucucAAucGccGcGucGcdTsdT	623	GCGACGCGGCGAUUGAGACdTsdT	92	4	102	9
460	auccAuAcuGcGGAAcucccdTsdT	624	GGAGUUCCGcAGuAUGGAUdTsdT	88	3	102	7
461	cgcAccucucuuuAcGcGGdTsdT	625	CCGCGuAAAGAGAGGUGCGdTsdT	78	1	102	10
462	caAcGAccGAccuuGAGGcdTsdT	626	GCCUcAAGGUCGGUCGUUGdTsdT	88	4	102	8
463	ccAuAcuGcGGAAcucccuAdTsdT	627	uAGGAGUUCCGcAGuAUGGdTsdT	85	3	102	5
464	ugAAucccGcGGAcGAcccdTsdT	628	GGGUCGUCCGCGGGAUUcAdTsdT	92	4	103	3
465	agAAcucccucGccucGcAdTsdT	629	UGCGAGGCGAGGGAGUUCUdTsdT	94	5	103	2
466	ggcGcAccucucuuuAcGcdTsdT	630	GCGuAAAGAGAGGUGCGCCdTsdT	97	7	103	10
467	gcGcAccucucuuuAcGcGdTsdT	631	CGCGuAAAGAGAGGUGCGCdTsdT	99	5	104	7

FIG. 2

468	gcuGAAucccGcGGAcGAcdTsdT	632	GUCGUCCGCGGGAUUcAGCdTsdT	84	2	104	3
469	cacuucGcuucAccucuGcdTsdT	633	GcAGAGGUGAAGCGAAGUGdTsdT	90	4	105	12
470	cucAAucGccGcGucGcAGdTsdT	634	CUGCGACGCGGCGAUUGAGdTsdT	99	3	105	14
471	ucccGucGGcGcuGAAuccdTsdT	635	GGAUUcAGCGCCGACGGGAdTsdT	91	3	106	7
472	cuGAAucccGcGGAcGAccdTsdT	636	GGUCGUCCGCGGGAUUcAGdTsdT	96	2	106	6
473	agAGucuAGAcucGuGGuGdTsdT	637	cACcACGAGUCuAGACUCUdTsdT	93	4	107	9
474	uccAuAcuGcGGAAcucccudTsdT	638	AGGAGUUCCGcAGuAUGGAdTsdT	91	4	107	7
475	gcGcuGAAucccGcGGAcGdTsdT	639	CGUCCGCGGGAUUcAGCGCdTsdT	90	3	108	3
476	aguGuGGAuucGcAcucccudTsdT	640	AGGAGUGCGAAUCcAcACUdTsdT	94	4	111	3
477	cccuGcucGuGuuAcAGGcdTsdT	641	GCCUGuAAcACGAGcAGGGdTsdT	99	11	111	10
478	gaAucccGcGGAcGAcccGdTsdT	642	CGGGUCGUCCGCGGGAUUCdTsdT	96	3	115	5
479	aaGcuGuGccuuGGGuGGcdTsdT	643	GCcACCcAAGGcAcAGCUUdTsdT	99	5	116	53
480	gcccuGcucGuGuuAcAGGdTsdT	644	CCUGuAAcACGAGcAGGGCdTsdT	96	5	116	11
481	gucccGucGGcGcuGAAucdTsdT	645	GAUUcAGCGCCGACGGGACdTsdT	93	2	118	4
482	aucuuAucAAcAcuuccGGdTsdT	646	CCGGAAGUGUUGAuAAGAUdTsdT	76	3	126	23
483	cuuAucAAcAcuuccGGAAdTsdT	647	UUCCGGAAGUGUUGAuAAGGdTsdT	39	6	42	3

FIG. 2

Table 3. Serum stability of dsRNAs targeting Hepatitis B Virus.

SEQ ID No. Pair	Mouse Serum		Human Serum		Cynomologous Serum	
	sense $t_{1/2}$ (hr)	antisense $t_{1/2}$ (hr)	sense $t_{1/2}$ (hr)	antisense $t_{1/2}$ (hr)	sense $t_{1/2}$ (hr)	antisense $t_{1/2}$ (hr)
321/485	26.4	0.5	>48	2.1	n.d.	n.d.
325/489	27.2	6.7	>48	8.8	n.d.	n.d.
350/514	11.3	2.6	>48	17.0	n.d.	n.d.
326/490	>48	11.7	>48	43.9	>48	5.5
324/488	>48	13.3	>48	44.7	>48	6.4
328/492	19.1	9.9	>48	>48	n.d.	n.d.
322/486	>48	14.5	>48	>48	>48	6.5
327/491	>48	16.0	>48	>48	>48	8.1

FIG. 3

Table 4. Core sequences of dsRNAs targeting Hepatitis B Virus gene and their modified counterparts.

core sequence				modified sequence			
SEQ ID No.	sense strand sequence (5′-3′)	SEQ ID No.	antisense strand sequence (5′-3′)	SEQ ID No.	sense strand sequence (5′-3′)	SEQ ID No.	antisense strand sequence (5′-3′)
1	CAAGGUAUGUUGCCCG UUU	157	AAACGGGCAACAUACCUUG	321	caAGGuAuGuuGcccGuuu dTsdT	485	AAACGGGcAAcAuACCUUGd TsdT
2	CUGUAGGCAUAAAUUG GUA	158	TACCAAUUUAUGCCUACAG	322	CfuGfuAfgGfcAfuAfaAfu UfgGfuAf(invdT)	486	pdTAfcCfaAfuUfuAfuGfcCfu AfcAfgdTsdT
3	UCUGCGGCGUUUUAUC AUA	159	UAUGAUAAAACGCCGCAGA	323	ucuGcGGcGuuuuAucAuA dTsdT	487	uAUGAuAAAACGCCGcAGAd TsdT
3	UCUGCGGCGUUUUAUC AUA	160	TAUGAUAAAACGCCGCAGA	324	UfcUfgCfgGfcGfuUfuUfa UfcAfuAf(invdT)	488	pdTAfuGfaUfaAfaAfcGfcCfg CfaGfadTsdT
4	ACCUCUGCCUAAUCAUC UC	161	GAGAUGAUUAGGCAGAGG U	325	accucuGccuAAucAucucd TsdT	489	GAGAUGAUuAGGcAGAGGU dTsdT
5	UUUACUAGUGCCAUUU GUA	162	TACAAAUGGCACUAGUAAA	326	UfuUfaCfuAfgUfgCfcAfu UfuGfuAf(invdT)	490	pdTAfcAfaAfuGfgCfaCfuAfg UfaAfadTsdT
6	ACCUCUGCCUAAUCAUC UA	163	TAGAUGAUUAGGCAGAGGU	327	AfcCfuCfuGfcCfuAfaUfcA fuCfuAf(invdT)	491	pdTAfgAfuGfaUfuAfgGfcAfg AfgGfudTsdT
7	CUGUAGGCAUAAAUUG GUC	164	GACCAAUUUAUGCCUACAG	328	cuGuAGGcAuAAAuuGGu cdTsdT	492	GACcAAUUuAUGCCuAcAGd TsdT
8	UGUCUGCGGCGUUUUA UCA	165	UGAUAAAACGCCGCAGACA	329	ugucuGcGGcGuuuuAucA dTsdT	493	UGAuAAAACGCCGcAGAcAd TsdT
8	UGUCUGCGGCGUUUUA UCA	166	TGAUAAAACGCCGCAGACA	330	UfgUfcUfgCfgGfcGfuUfu UfaUfcAf(invdT)	494	pdTGfaUfaAfaAfcGfcCfgCfa GfaCfadTsdT
9	UACUAGUGCCAUUUGU UCA	167	UGAACAAAUGGCACUAGUA	331	uacuAGuGccAuuuGuucA dTsdT	495	UGAAcAAAUGGcACuAGuAd TsdT
9	UACUAGUGCCAUUUGU UCA	168	TGAACAAAUGGCACUAGUA	332	UfaCfuAfgUfgCfcAfuUfu GfuUfcAf(invdT)	496	pdTGfaAfcAfaAfuGfgCfaCfu AfgUfadTsdT
10	CAACUUUUUCACCUCUG	169	TGCAGAGGUGAAAAAGUUG	333	CfaAfcUfuUfuUfcAfcCfuC	497	pdTGfcAfgAfgGfuGfaAfaAfa

FIG. 4

	CA				fuGfcAf(invdT)		GfuUfgdTsdT
11	CCAUUUGUUCAGUGGU UCG	170	CGAACCACUGAACAAAUGG	334	ccAuuuGuucAGuGGuucG dTsdT	498	CGAACcACUGAAcAAAUGGd TsdT
12	CCAAGUGUUUGCUGAC GCA	171	UGCGUCAGCAAACACUUGG	335	ccAAGuGuuuGcuGAcGc AdTsdT	499	UGCGUcAGcAAAcACUUGGd TsdT
12	CCAAGUGUUUGCUGAC GCA	172	TGCGUCAGCAAACACUUGG	336	CfcAfaGfuGfuUfuGfcUfg AfcGfcAf(invdT)	500	pdTGfcGfuCfaGfcAfaAfcAfc UfuGfgdTsdT
13	CCAUUUGUUCAGUGGU UCA	173	TGAACCACUGAACAAAUGG	337	CfcAfuUfuGfuUfcAfgUfg GfuUfcAf(invdT)	501	pdTGfaAfcCfaCfuGfaAfcAfa AfuGfgdTsdT
14	UUUACUAGUGCCAUUU GUU	174	AACAAAUGGCACUAGUAAA	338	uuuAcuAGuGccAuuuGuu dTsdT	502	AAcAAAUGGcACuAGuAAAd TsdT
15	CACCUCUGCCUAAUCAU CA	175	TGAUGAUUAGGCAGAGGU G	339	CfaCfcUfcUfgCfcUfaAfuC faUfcAf(invdT)	503	pdTGfaUfgAfuUfaGfgCfaGfa GfgUfgdTsdT
16	CUGGCUCAGUUUACUA GUG	176	CACUAGUAAACUGAGCCAG	340	cuGGcucAGuuuAcuAGu GdTsdT	504	cACuAGuAAACUGAGCcAGd TsdT
17	CAAGGUAUGUUGCCCG UUA	177	TAACGGGCAACAUACCUUG	341	CfaAfgGfuAfuGfuUfgCfc CfgUfuAf(invdT)	505	pdTAfaCfgGfgCfaAfcAfuAfcC fuUfgdTsdT
18	CUGGCUCAGUUUACUA GUA	178	TACUAGUAAACUGAGCCAG	342	CfuGfgCfuCfaGfuUfuAfc UfaGfuAf(invdT)	506	pdTAfcUfaGfuAfaAfcUfgAfg CfcAfgdTsdT
19	GAGGCUGUAGGCAUAA AUU	179	AAUUUAUGCCUACAGCCUC	343	gaGGcuGuAGGcAuAAAu udTsdT	507	AAUUuAUGCCuAcAGCCUCd TsdT
20	CAGUUUACUAGUGCCAU UU	180	AAAUGGCACUAGUAAACUG	344	caGuuuAcuAGuGccAuuu dTsdT	508	AAAUGGcACuAGuAAACUGd TsdT
21	AGGUAUGUUGCCCGUU UGU	181	ACAAACGGGCAACAUACCU	345	agGuAuGuuGcccGuuuGu dTsdT	509	AcAAACGGGcAAcAuACCUd TsdT
22	UAUGUUGCCCGUUUGU CCA	182	UGGACAAACGGGCAACAUA	346	UfaUfgUfuGfcCfcGfuUfu GfuCfcAf(invdT)	510	pdTGfgAfcAfaAfcGfgGfcAfa CfaUfadTsdT
23	GAGGCUGUAGGCAUAA AUA	183	TAUUUAUGCCUACAGCCUC	347	GfaGfgCfuGfuAfgGfcAfu AfaAfuAf(invdT)	511	pdTAfuUfuAfuGfcCfuAfcAfg CfcUfcdTsdT
24	GUCUGCGGCGUUUUAU CAU	184	AUGAUAAAACGCCGCAGAC	348	gucuGcGGcGuuuuAucAu dTsdT	512	AUGAuAAAACGCCGcAGACd TsdT

FIG. 4

25	CAACUUUUUCACCUCUG CC	185	GGCAGAGGUGAAAAAGUU G	349	caAcuuuuucAccucuGccd TsdT	513	GGcAGAGGUGAAAAAGUUG dTsdT
26	CCGUGUGCACUUCGCUU CA	186	UGAAGCGAAGUGCACACGG	350	ccGuGuGcAcuucGcuucA dTsdT	514	UGAAGCGAAGUGcAcACGG dTsdT
26	CCGUGUGCACUUCGCUU CA	187	TGAAGCGAAGUGCACACGG	351	CfcGfuGfuGfcAfcUfuCfgC fuUfcAf(invdT)	515	pdTGfaAfgCfgAfaGfuGfcAfc AfcGfgdTsdT
27	UCAAGGUAUGUUGCCC GUA	188	TACGGGCAACAUACCUUGA	352	UfcAfaGfgUfaUfgUfuGfc CfcGfuAf(invdT)	516	pdTAfcGfgGfcAfaCfaUfaCfc UfuGfadTsdT
28	CAGUUUACUAGUGCCAU UA	189	TAAUGGCACUAGUAAACUG	353	CfaGfuUfuAfcUfaGfuGfc CfaUfuAf(invdT)	517	pdTAfaUfgGfcAfcUfaGfuAfa AfcUfgdTsdT
29	UGGUGGACUUCUCUCA AUU	190	AAUUGAGAGAAGUCCACCA	354	ugGuGGAcuucucucAAuu dTsdT	518	AAUUGAGAGAAGUCcACcAd TsdT
30	AGGUAUGUUGCCCGUU UGA	191	TCAAACGGGCAACAUACCU	355	AfgGfuAfuGfuUfgCfcCfg UfuUfgAf(invdT)	519	pdTCfaAfaCfgGfgCfaAfcAfu AfcCfudTsdT
31	CUGCUCGUGUUACAGGC GG	192	CCGCCUGUAACACGAGCAG	356	cuGcucGuGuuAcAcGGcG GdTsdT	520	CCGCCUGuAAcAcGAGcAGd TsdT
32	UAUGUUGCCCGUUUGU CCU	193	AGGACAAACGGGCAACAUA	357	uauGuuGcccGuuuGuccu dTsdT	521	AGGAcAAACGGGcAAcAuAd TsdT
33	UCAAGGUAUGUUGCCC GUU	194	AACGGGCAACAUACCUUGA	358	ucAAGGuAuGuuGcccGu udTsdT	522	AACGGGcAAcAuACCUUGAd TsdT
34	UCUUAUCAACACUUCCG GA	195	UCCGGAAGUGUUGAUAAG A	359	ucuuAucAAcAcuuccGGA dTsdT	523	UCCGGAAGUGUUGAuAAGA dTsdT
34	UCUUAUCAACACUUCCG GA	196	TCCGGAAGUGUUGAUAAGA	360	UfcUfuAfuCfaAfcAfcUfuC fcGfgAf(invdT)	524	pdTCfcGfgAfaGfuGfuUfgAfu AfaGfadTsdT
35	CACCUCUGCCUAAUCAU CU	197	AGAUGAUUAGGCAGAGGU G	361	caccucuGccuAAucAucud TsdT	525	AGAUGAUuAGGcAGAGGUG dTsdT
36	AUAAGAGGACUCUUGG ACU	198	AGUCCAAGAGUCCUCUUAU	362	auAAGAGGAcucuuGGAc udTsdT	526	AGUCcAAGAGUCCUCUuAU dTsdT
37	GUCUGCGGCGUUUUAU CAA	199	TUGAUAAAACGCCGCAGAC	363	GfuCfuGfcGfgCfgUfuUfu AfuCfaAf(invdT)	527	pdTUfgAfuAfaAfaCfgCfcGfc AfgAfcdTsdT
38	GGCGCUGAAUCCCGCGG	200	GUCCGCGGGAUUCAGCGCC	364	ggcGcuGAAucccGcGGAc	528	GUCCGCGGGAUUcAGCGCC

FIG. 4

	AC			dTsdT		dTsdT
39	CGCGUCGCAGAAGAUCUCA	201	UGAGAUCUUCUGCGACGCG	365	cgcGucGcAGAAGAucucA dTsdT	529	UGAGAUCUUCUGCGACGCG dTsdT
40	AAUGUCAACGACCGACCUU	202	AAGGUCGGUCGUUGACAU U	366	aauGucAAcGAccGAccuu dTsdT	530	AAGGUCGGUCGUUGAcAUU dTsdT
41	GCUCAGUUUACUAGUGCCA	203	UGGCACUAGUAAACUGAGC	367	gcucAGuuuAcuAGuGccA dTsdT	531	UGGcACuAGuAAACUGAGCd TsdT
42	UGGUGGACUUCUCUCAAUA	204	TAUUGAGAGAAGUCCACCA	368	UfgGfuGfgAfcUfuCfuCfu CfaAfuAf(invdT)	532	pdTAfuUfgAfgAfgAfaGfuCfc AfcCfadTsdT
43	AUCGCCGCGUCGCAGAAGA	205	UCUUCUGCGACGCGGCGAU	369	aucGccGcGucGcAGAAGA dTsdT	533	UCUUCUGCGACGCGGCGAU dTsdT
44	GCCAUUUGUUCAGUGGUUC	206	GAACCACUGAACAAAUGGC	370	gccAuuuGuucAGuGGuuc dTsdT	534	GAACcACUGAAcAAAUGGCd TsdT
45	CGAUCCAUACUGCGGAACU	207	AGUUCCGCAGUAUGGAUCG	371	cgAuccAuAcuGcGGAAcu dTsdT	535	AGUUCCGcAGuAUGGAUCG dTsdT
46	UCACCUCUGCCUAAUCAUC	208	GAUGAUUAGGCAGAGGUGA	372	ucAccucuGccuAAucAucd TsdT	536	GAUGAUuAGGcAGAGGUGA dTsdT
47	GUGGACUUCUCUCAAUUUU	209	AAAAUUGAGAGAAGUCCAC	373	guGGAcuucucucAAuuuu dTsdT	537	AAAAUUGAGAGAAGUCcAC dTsdT
48	GGGUCACCAUAUUCUUGGG	210	CCCAAGAAUAUGGUGACCC	374	ggGucAccAuAuucuuGGG dTsdT	538	CCcAAGAAuAUGGUGACCCd TsdT
49	GCCGCGUCGCAGAAGAUCU	211	AGAUCUUCUGCGACGCGGC	375	gccGcGucGcAGAAGAucu dTsdT	539	AGAUCUUCUGCGACGCGGC dTsdT
50	UCAAUCGCCGCGUCGCAGA	212	UCUGCGACGCGGCGAUUGA	376	ucAAucGccGcGucGcAGA dTsdT	540	UCUGCGACGCGGCGAUUGA dTsdT
51	UGGAUGUGUCUGCGGCGUU	213	AACGCCGCAGACACAUCCA	377	ugGAuGuGucuGcGGcGu udTsdT	541	AACGCCGcAGAcAcAUCcAdT sdT
52	UACUGUUCAAGCCUCCAAG	214	CUUGGAGGCUUGAACAGUA	378	uacuGuucAAGccuccAAG dTsdT	542	CUUGGAGGCUUGAAcAGuA dTsdT
53	GUUUACUAGUGCCAUUGU	215	ACAAAUGGCACUAGUAAAC	379	guuuAcuAGuGccAuuuGu dTsdT	543	AcAAAUGGcACuAGuAAACd TsdT

FIG. 4

54	ACUAGUGCCAUUUGUU CAG	216	CUGAACAAAUGGCACUAGU	380	acuAGuGccAuuuGuucAG dTsdT	544	CUGAAcAAAUGGcACuAGUd TsdT
55	CCGCGUCGCAGAAGAUC UC	217	GAGAUCUUCUGCGACGCGG	381	ccGcGucGcAGAAGAucuc dTsdT	545	GAGAUCUUCUGCGACGCGG dTsdT
56	UAUCUUAUCAACACUUC CG	218	CGGAAGUGUUGAUAAGAU A	382	uaucuuAucAAcAcuuccGd TsdT	546	CGGAAGUGUUGAuAAGAuA dTsdT
57	GGCCAAAAUUCGCAGUC CC	219	GGGACUGCGAAUUUUGGCC	383	ggccAAAAuucGcAGucccd TsdT	547	GGGACUGCGAAUUUUGGCC dTsdT
58	UUCACCUCUGCCUAAUC AU	220	AUGAUUAGGCAGAGGUGA A	384	uucAccucuGccuAAucAud TsdT	548	AUGAUuAGGcAGAGGUGAA dTsdT
59	CUCAGUUUACUAGUGCC AU	221	AUGGCACUAGUAAACUGAG	385	cucAGuuuAcuAGuGccAu dTsdT	549	AUGGcACuAGuAAACUGAG dTsdT
60	UGUUGCCCGUUUGUCC UCU	222	AGAGGACAAACGGGCAACA	386	uguuGcccGuuuGucccucud TsdT	550	AGAGGAcAAACGGGcAAcAd TsdT
61	UAGUGCCAUUUGUUCA GUG	223	CACUGAACAAAUGGCACUA	387	uaGuGccAuuuGuucAGu GdTsdT	551	cACUGAAcAAAUGGcACuAd TsdT
62	AGGCUGUAGGCAUAAA UUG	224	CAAUUUAUGCCUACAGCCU	388	agGcuGuAGGcAuAAAuu GdTsdT	552	cAAUUuAUGCCuAcAGCCUd TsdT
63	AUGUGUCUGCGGCGUU UUA	225	UAAAACGCCGCAGACACAU	389	auGuGucuGcGGcGuuuu AdTsdT	553	uAAAACGCCGcAGAcAcAUd TsdT
63	AUGUGUCUGCGGCGUU UUA	226	TAAAACGCCGCAGACACAU	390	AfuGfuGfuCfuGfcGfgCfg UfuUfuAf(invdT)	554	pdTAfaAfaCfgCfcGfcAfgAfcA fcAfudTsdT
64	ACUUCGCUUCACCUCUG CA	227	UGCAGAGGUGAAGCGAAGU	391	acuucGcuucAccucuGcAd TsdT	555	UGcAGAGGUGAAGCGAAGU dTsdT
65	CGUGUGCACUUCGCUUC AC	228	GUGAAGCGAAGUGCACACG	392	cguGuGcAcuucGcuucAcd TsdT	556	GUGAAGCGAAGUGcAcACG dTsdT
66	GUGGUGGACUUCUCUC AAU	229	AUUGAGAGAAGUCCACCAC	393	guGGuGGAcuucucucAAu dTsdT	557	AUUGAGAGAAGUCcAcCAcd TsdT
67	UGUGUCUGCGGCGUUU UAU	230	AUAAAACGCCGCAGACACA	394	uguGucuGcGGcGuuuuAu dTsdT	558	AuAAAACGCCGcAGAcAcAdT sdT
68	AAGGUAUGUUGCCCGU	231	CAAACGGGCAACAUACCUU	395	aaGGuAuGuuGcccGuuu	559	cAAACGGGcAAcAuACCUUd

FIG. 4

	UUG			GdTsdT		TsdT
69	UCAACGACCGACCUUGAGG	232	CCUCAAGGUCGGUCGUUGA	396	ucAAcGAccGAccuuGAGG dTsdT	560	CCUcAAGGUCGGUCGUUGA dTsdT
70	CAUAAGAGGACUCUUGGAC	233	GUCCAAGAGUCCUCUUAUG	397	cauAAGAGGAcucuuGGA cdTsdT	561	GUCcAAGAGUCCUCUuAUG dTsdT
71	GUCAACGACCGACCUUGAG	234	CUCAAGGUCGGUCGUUGAC	398	gucAAcGAccGAccuuGAG dTsdT	562	CUcAAGGUCGGUCGUUGAC dTsdT
72	AUAUUCUUGGGAACAAGAG	235	CUCUUGUUCCCAAGAAUAU	399	auAuucuuGGGAAcAAGA GdTsdT	563	CUCUUGUUCCcAAGAAuAU dTsdT
73	UGCUCGUGUUACAGGCGGG	236	CCCGCCUGUAACACGAGCA	400	ugcucGuGuuAcAGGcGG GdTsdT	564	CCCGCCUGuAAcACGAGcAd TsdT
74	CAAUCGCCGCGUCGCAGAA	237	UUCUGCGACGCGGCGAUUG	401	caAucGccGcGucGcAGAA dTsdT	565	UUCUGCGACGCGGCGAUUG dTsdT
75	ACUGUUCAAGCCUCCAAGC	238	GCUUGGAGGCUUGAACAGU	402	acuGuucAAGccuccAAGc dTsdT	566	GCUUGGAGGCUUGAAcAGU dTsdT
76	CGCCGCGUCGCAGAAGAUC	239	GAUCUUCUGCGACGCGGCG	403	cgccGcGucGcAGAAGAuc dTsdT	567	GAUCUUCUGCGACGCGGCG dTsdT
77	CAUUUGUUCAGUGGUUCGU	240	ACGAACCACUGAACAAAUG	404	cauuuGuucAGuGGuucGu dTsdT	568	ACGAACcACUGAAcAAAUGd TsdT
78	CGCUGAAUCCCGCGGACGA	241	UCGUCCGCGGGAUUCAGCG	405	cgcuGAAucccGcGGAcGA dTsdT	569	UCGUCCGCGGGAUUcAGCG dTsdT
79	UGGGUCACCAUAUUCUUGG	242	CCAAGAAUAUGGUGACCCA	406	ugGGucAccAuAuucuuGG dTsdT	570	CcAAGAAuAUGGUGACCcAd TsdT
80	UCCUCUGCCGAUCCAUACU	243	AGUAUGGAUCGGCAGAGGA	407	uccucuGccGAuccAuAcud TsdT	571	AGuAUGGAUCGGcAGAGGA dTsdT
81	AUGUCAACGACCGACCUUG	244	CAAGGUCGGUCGUUGACAU	408	auGucAAcGAccGAccuuG dTsdT	572	cAAGGUCGGUCGUUGAcAU dTsdT
82	CCUCUGCCUAAUCAUCUCA	245	UGAGAUGAUUAGGCAGAGG	409	ccucuGccuAAucAucucAd TsdT	573	UGAGAUGAUuAGGcAGAGG dTsdT
83	ACCGUGUGCACUUCGCUUC	246	GAAGCGAAGUGCACACGGU	410	accGuGuGcAcuucGcuuc dTsdT	574	GAAGCGAAGUGcAcACGGU dTsdT

FIG. 4

84	UGCCGAUCCAUACUGCGGA	247	UCCGCAGUAUGGAUCGGCA	411	ugccGAuccAuAcuGcGGA dTsdT	575	UCCGcAGuAUGGAUCGGcA dTsdT
85	CAGAGUCUAGACUCGUGGU	248	ACCACGAGUCUAGACUCUG	412	caGAGucuAGAcucGuGG udTsdT	576	ACcACGAGUCuAGACUCUGd TsdT
86	CUGUUCAAGCCUCCAAGCU	249	AGCUUGGAGGCUUGAACAG	413	cuGuucAAGccuccAAGcu dTsdT	577	AGCUUGGAGGCUUGAAcAG dTsdT
87	GGAGGCUGUAGGCAUAAAU	250	AUUUAUGCCUACAGCCUCC	414	ggAGGcuGuAGGcAuAAA udTsdT	578	AUUuAUGCCuAcAGCCUCCd TsdT
88	AGGAGGCUGUAGGCAUAAA	251	UUUAUGCCUACAGCCUCCU	415	agGAGGcuGuAGGcAuAA AdTsdT	579	UUuAUGCCuAcAGCCUCCUd TsdT
89	GGUGGACUUCUCUCAAUUU	252	AAAUUGAGAGAAGUCCACC	416	gguGGAcuucucucAAuuu dTsdT	580	AAAUUGAGAGAAGUCcACC dTsdT
90	GCAACUUUUUCACCUCUGC	253	GCAGAGGUGAAAAAGUUGC	417	gcAAcuuuuucAccucuGcd TsdT	581	GcAGAGGUGAAAAAGUUGC dTsdT
91	CUGCUCGUGUUACAGGCGA	254	TCGCCUGUAACACGAGCAG	418	CfuGfcUfcGfuGfuUfaCfa GfgCfgAf(invdT)	582	pdTCfgCfcUfgUfaAfcAfcGfa GfcAfgdTsdT
92	CUAGUGCCAUUUGUUCAGU	255	ACUGAACAAAUGGCACUAG	419	cuAGuGccAuuuGuucAGu dTsdT	583	ACUGAAcAAAUGGcACuAGd TsdT
93	CUGCCGAUCCAUACUGCGG	256	CCGCAGUAUGGAUCGGCAG	420	cuGccGAuccAuAcuGcGG dTsdT	584	CCGcAGuAUGGAUCGGcAGd TsdT
94	GUGUGCACUUCGCUUCACC	257	GGUGAAGCGAAGUGCACAC	421	guGuGcAcuucGcuucAccd TsdT	585	GGUGAAGCGAAGUGcAcAC dTsdT
95	GCUCGUGUUACAGGCGGGC	258	GCCCGCCUGUAACACGAGC	422	gcucGuGuuAcAGGcGGG cdTsdT	586	GCCCGCCUGuAAcACGAGCd TsdT
96	CCUAUCUUAUCAACACUUC	259	GAAGUGUUGAUAAGAUAG G	423	ccuAucuuAucAAcAcuucd TsdT	587	GAAGUGUUGAuAAGAuAGG dTsdT
97	UCUCAAUCGCCGCGUCGCA	260	UGCGACGCGGCGAUUGAGA	424	ucucAAucGccGcGucGcA dTsdT	588	UGCGACGCGGCGAUUGAGA dTsdT
98	GCCCGUCUGUGCCUUCUCA	261	UGAGAAGGCACAGACGGGC	425	gcccGucuGuGccuucucAd TsdT	589	UGAGAAGGcAcAGACGGGC dTsdT
99	CUAUCUUAUCAACACUUU	262	GGAAGUGUUGAUAAGAUA	426	cuAucuuAucAAcAcuuccd	590	GGAAGUGUUGAuAAGAuAG

FIG. 4

	CC		G		TsdT		dTsdT
100	AUGUUGCCCGUUUGUC CUC	263	GAGGACAAACGGGCAACAU	427	auGuuGcccGuuuGuccuc dTsdT	591	GAGGAcAAACGGGcAAcAUd TsdT
101	GUAUGUUGCCCGUUUG UCC	264	GGACAAACGGGCAACAUAC	428	guAuGuuGcccGuuuGucc dTsdT	592	GGAcAAACGGGcAAcAuACd TsdT
102	CUUCGCUUCACCUCUGC AC	265	GUGCAGAGGUGAAGCGAAG	429	cuucGcuucAccucuGcAcd TsdT	593	GUGcAGAGGUGAAGCGAAG dTsdT
103	UGUGCACUUCGCUUCAC CU	266	AGGUGAAGCGAAGUGCACA	430	uguGcAcuucGcuucAccud TsdT	594	AGGUGAAGCGAAGUGcAcA dTsdT
104	GCCAAAAUUCGCAGUCC CG	267	CGGGACUGCGAAUUUUGGC	431	gccAAAAuucGcAGucccG dTsdT	595	CGGGACUGCGAAUUUUGGC dTsdT
105	CCUGCUCGUGUUACAGG CG	268	CGCCUGUAACACGAGCAGG	432	ccuGcucGuGuuAcAGGcG dTsdT	596	CGCCUGuAAcACGAGcAGGd TsdT
106	UGGAGUGUGGAUUCGC ACU	269	AGUGCGAAUCCACACUCCA	433	ugGAGuGuGGAuucGcAc udTsdT	597	AGUGCGAAUCcAcACUCcAd TsdT
107	AACGACCGACCUUGAGG CA	270	UGCCUCAAGGUCGGUCGUU	434	aacGAccGAccuuGAGGcA dTsdT	598	UGCCUcAAGGUCGGUCGUU dTsdT
108	ACAGAGUCUAGACUCGU GG	271	CCACGAGUCUAGACUCUGU	435	acAGAGucuAGAcucGuG GdTsdT	599	CcACGAGUCuAGACUCUGU dTsdT
109	AAUCGCCGCGUCGCAGA AG	272	CUUCUGCGACGCGGCGAUU	436	aaucGccGcGucGcAGAAG dTsdT	600	CUUCUGCGACGCGGCGAUU dTsdT
110	GGUAUGUUGCCCGUUU GUC	273	GACAAACGGGCAACAUACC	437	gguAuGuuGcccGuuuGuc dTsdT	601	GAcAAACGGGcAAcAuACCd TsdT
111	GCCGAUCCAUACUGCGG AA	274	UUCCGCAGUAUGGAUCGGC	438	gccGAuccAuAcuGcGGAA dTsdT	602	UUCCGcAGuAUGGAUCGGC dTsdT
112	GCCCUAUCUUAUCAACA CU	275	AGUGUUGAUAAGAUAGGG C	439	gcccuAucuuAucAAcAcud TsdT	603	AGUGUUGAuAAGAuAGGGC dTsdT
113	AGUUUACUAGUGCCAU UUG	276	CAAAUGGCACUAGUAAACU	440	aguuuAcuAGuGccAuuuG dTsdT	604	cAAAUGGcACuAGuAAACUd TsdT
114	UGUCAACGACCGACCUU GA	277	UCAAGGUCGGUCGUUGACA	441	ugucAAcGAccGAccuuGA dTsdT	605	UcAAGGUCGGUCGUUGAcA dTsdT

FIG. 4

115	ACUUCUCUCAAUUUUCUAG	278	CUAGAAAAUUGAGAGAAGU	442	acuucucucAAuuuucuAG dTsdT	606	CuAGAAAAUUGAGAGAAGU dTsdT
116	GCGCGGGACGUCCUUUGUC	279	GACAAAGGACGUCCCGCGC	443	gcGcGGGAcGuccuuuGuc dTsdT	607	GAcAAAGGACGUCCCGCGCd TsdT
117	UCUAGACUCGUGGUGGACU	280	AGUCCACCACGAGUCUAGA	444	ucuAGAcucGuGGuGGAc udTsdT	608	AGUCcACcACGAGUCuAGAd TsdT
118	GAUCCAUACUGCGGAACUC	281	GAGUUCCGCAGUAUGGAUC	445	gauccAuAcuGcGGAAcuc dTsdT	609	GAGUUCCGcAGuAUGGAUC dTsdT
119	CUCUGCCGAUCCAUACUGC	282	GCAGUAUGGAUCGGCAGAG	446	cucuGccGAuccAuAcuGcd TsdT	610	GcAGuAUGGAUCGGcAGAG dTsdT
120	UCUGCCGAUCCAUACUGCG	283	CGCAGUAUGGAUCGGCAGA	447	ucuGccGAuccAuAcuGcG dTsdT	611	CGcAGuAUGGAUCGGcAGA dTsdT
121	CCUCUGCCGAUCCAUACUG	284	CAGUAUGGAUCGGCAGAGG	448	ccucuGccGAuccAuAcuGd TsdT	612	cAGuAUGGAUCGGcAGAGG dTsdT
122	GCACCUCUCUUUACGCGGU	285	ACCGCGUAAAGAGAGGUGC	449	gcAccucucuuuAcGcGGud TsdT	613	ACCGCGuAAAGAGAGGUGC dTsdT
123	AAGAACUCCCUCGCCUCGC	286	GCGAGGCGAGGGAGUUCU U	450	aaGAAcucccucGccucGcd TsdT	614	GCGAGGCGAGGGAGUUCU UdTsdT
124	GAACUCCCUCGCCUCGCAG	287	CUGCGAGGCGAGGGAGUUC	451	gaAcucccucGccucGcAGd TsdT	615	CUGCGAGGCGAGGGAGUUC dTsdT
125	UCUCUCAAUUUUCUAGGGC	288	GCCCUAGAAAAUUGAGAGA	452	ucucucAAuuuuucuAGGGc dTsdT	616	GCCCuAGAAAAUUGAGAGA dTsdT
126	GGGCGCACCUCUCUUUACG	289	CGUAAAGAGAGGUGCGCCC	453	ggGcGcAccucucuuuAcGd TsdT	617	CGuAAAGAGAGGUGCGCCC dTsdT
127	CCGAUCCAUACUGCGGAAC	290	GUUCCGCAGUAUGGAUCGG	454	ccGAuccAuAcuGcGGAAc dTsdT	618	GUUCCGcAGuAUGGAUCGG dTsdT
128	AACUCCCUCGCCUCGCAGA	291	UCUGCGAGGCGAGGGAGU U	455	aacucccucGccucGcAGAd TsdT	619	UCUGCGAGGCGAGGGAGU UdTsdT
129	CUCCUCUGCCGAUCCAUAC	292	GUAUGGAUCGGCAGAGGA G	456	cuccucuGccGAuccAuAcd TsdT	620	GuAUGGAUCGGcAGAGGAG dTsdT
130	GGAGUGUGGAUUCGCAGAG	293	GAGUGCGAAUCCACACUCC	457	ggAGuGuGGAuucGcAcuc	621	GAGUGCGAAUCcAcACUCCd

FIG. 4

	CUC				dTsdT		TsdT
131	CGGGCGCACCUCUCUUUAC	294	GUAAAGAGAGGUGCGCCCG	458	cgGGcGcAccucucuuuAcd TsdT	622	GuAAAGAGAGGUGCGCCCG dTsdT
132	GUCUCAAUCGCCGCGUCGC	295	GCGACGCGGCGAUUGAGAC	459	gucucAAucGccGcGucGcd TsdT	623	GCGACGCGGCGAUUGAGAC dTsdT
133	AUCCAUACUGCGGAACUCC	296	GGAGUUCCGCAGUAUGGAU	460	auccAuAcuGcGGAAcucc dTsdT	624	GGAGUUCCGcAGuAUGGAU dTsdT
134	CGCACCUCUCUUUACGCGG	297	CCGCGUAAAGAGAGGUGCG	461	cgcAccucucuuuAcGcGGd TsdT	625	CCGCGuAAAGAGAGGUGCG dTsdT
135	CAACGACCGACCUUGAGGC	298	GCCUCAAGGUCGGUCGUUG	462	caAcGAccGAccuuGAGGc dTsdT	626	GCCUcAAGGUCGGUCGUUG dTsdT
136	CCAUACUGCGGAACUCCUA	299	UAGGAGUUCCGCAGUAUGG	463	ccAuAcuGcGGAAcuccuA dTsdT	627	uAGGAGUUCCGcAGuAUGG dTsdT
137	UGAAUCCCGCGGACGACCC	300	GGGUCGUCCGCGGGAUUCA	464	ugAAucccGcGGAcGAccc dTsdT	628	GGGUCGUCCGCGGGAUUcA dTsdT
138	AGAACUCCCUCGCCUCGCA	301	UGCGAGGCGAGGGAGUUCU	465	agAAcucccucGccucGcAd TsdT	629	UGCGAGGCGAGGGAGUUC UdTsdT
139	GGCGCACCUCUCUUUACGC	302	GCGUAAAGAGAGGUGCGCC	466	ggcGcAccucucuuuAcGcd TsdT	630	GCGuAAAGAGAGGUGCGCC dTsdT
140	GCGCACCUCUCUUUACGCG	303	CGCGUAAAGAGAGGUGCGC	467	gcGcAccucucuuuAcGcGd TsdT	631	CGCGuAAAGAGAGGUGCGC dTsdT
141	GCUGAAUCCCGCGGACGAC	304	GUCGUCCGCGGGAUUCAGC	468	gcuGAAucccGcGGAcGAc dTsdT	632	GUCGUCCGCGGGAUUcAGC dTsdT
142	CACUUCGCUUCACCUCUGC	305	GCAGAGGUGAAGCGAAGUG	469	cacuucGcuucAccucuGcd TsdT	633	GcAGAGGUGAAGCGAAGUG dTsdT
143	CUCAAUCGCCGCGUCGCAG	306	CUGCGACGCGGCGAUUGAG	470	cucAAucGccGcGucGcAG dTsdT	634	CUGCGACGCGGCGAUUGAG dTsdT
144	UCCCGUCGGCGCUGAAUCC	307	GGAUUCAGCGCCGACGGGA	471	ucccGucGGcGcuGAAucc dTsdT	635	GGAUUcAGCGCCGACGGGA dTsdT
145	CUGAAUCCCGCGGACGACC	308	GGUCGUCCGCGGGAUUCAG	472	cuGAAucccGcGGAcGAcc dTsdT	636	GGUCGUCCGCGGGAUUcAG dTsdT

FIG. 4

146	AGAGUCUAGACUCGUG GUG	309	CACCACGAGUCUAGACUCU	473	agAGucuAGAcucGuGGu GdTsdT	637	cACcACGAGUCuAGACUCUd TsdT
147	UCCAUACUGCGGAACUC CU	310	AGGAGUUCCGCAGUAUGGA	474	uccAuAcuGcGGAAcuccu dTsdT	638	AGGAGUUCCGcAGuAUGGA dTsdT
148	GCGCUGAAUCCCGCGGA CG	311	CGUCCGCGGGAUUCAGCGC	475	gcGcuGAAucccGcGGAcG dTsdT	639	CGUCCGCGGGAUUcAGCGC dTsdT
149	AGUGUGGAUUCGCACU CCU	312	AGGAGUGCGAAUCCACACU	476	aguGuGGAuucGcAcuccu dTsdT	640	AGGAGUGCGAAUCcAcACU dTsdT
150	CCCUGCUCGUGUUACAG GC	313	GCCUGUAACACGAGCAGGG	477	cccuGcucGuGuuAcAGGc dTsdT	641	GCCUGuAAcACGAGcAGGGd TsdT
151	GAAUCCCGCGGACGACC CG	314	CGGGUCGUCCGCGGGAUUC	478	gaAucccGcGGAcGAcccG dTsdT	642	CGGGUCGUCCGCGGGAUUC dTsdT
152	AAGCUGUGCCUUGGGU GGC	315	GCCACCCAAGGCACAGCUU	479	aaGcuGuGccuuGGGuGG cdTsdT	643	GCcACCcAAGGcAcAGCUUd TsdT
153	GCCCUGCUCGUGUUACA GG	316	CCUGUAACACGAGCAGGGC	480	gcccuGcucGuGuuAcAGG dTsdT	644	CCUGuAAcACGAGcAGGGCd TsdT
154	GUCCCGUCGGCGCUGAA UC	317	GAUUCAGCGCCGACGGGAC	481	gucccGucGGcGcuGAAuc dTsdT	645	GAUUcAGCGCCGACGGGAC dTsdT
155	AUCUUAUCAACACUUCC GG	318	CCGGAAGUGUUGAUAAGA U	482	aucuuAucAAcAcuuccGG dTsdT	646	CCGGAAGUGUUGAuAAGAU dTsdT
156	CUUAUCAACACUUCCGG AA	319	UUCCGGAAGUGUUGAUAA G	483	cuuAucAAcAcuuccGGAA dTsdT	647	UUCCGGAAGUGUUGAuAAG dTsdT
156	CUUAUCAACACUUCCGG AA	320	TUCCGGAAGUGUUGAUAAG	484	CfuUfaUfcAfaCfaCfuUfcC fgGfaAf(invdT)	648	pdTUfcCfgGfaAfgUfgUfuGfa UfaAfgdTsdT

FIG. 4

Table 5. Target site sequences of dsRNAs targeting Hepatitis B Virus

position of 17mer in acc. AM282986.1	Unmodified dsRNAs of table 1		Modified dsRNAs of table 2		17mer target site sequence (5'-3')	genotype coverage [%]			
	SEQ ID No. pair	SEQ ID No. pair	SEQ ID No. pair	SEQ ID No. pair		A (n=332)	B (n=615)	C (n=1332)	D (n=475)
456	1/157	17/177	321/485	341/505	AAGGUAUGUUGCCCGUU	91.3	94.3	94.9	78.3
383	3/159	3/160	323/487	324/488	CUGCGGCGUUUUAUCAU	96.7	95.9	95.9	94.3
1828	4/161	6/163	325/489	327/491	CCUCUGCCUAAUCAUCU	95.5	81.8	96.8	92.8
1782	7/164	2/158	328/492	322/486	UGUAGGCAUAAAUUGGU	97.9	96.1	95.9	97.5
381	8/165	8/166	329/493	330/494	GUCUGCGGCGUUUUAUC	96.4	84.9	94.0	93.9
679	9/167	9/168	331/495	332/496	ACUAGUGCCAUUUGUUC	96.4	90.4	94.9	94.7
687	11/170	13/173	334/498	337/501	CAUUUGUUCAGUGGUUC	96.7	90.4	96.1	97.7
1177	12/171	12/172	335/499	336/500	CAAGUGUUUGCUGACGC	91.3	95.9	91.8	77.5
677	14/174	5/162	338/502	326/490	UUACUAGUGCCAUUUGU	95.5	88.9	94.2	94.7
668	16/176	18/178	340/504	342/506	UGGCUCAGUUUACUAGU	97.0	94.6	91.0	94.7
1778	19/179	23/183	343/507	347/511	AGGCUGUAGGCAUAAAU	97.9	95.8	96.2	97.5
674	20/180	28/189	344/508	353/517	AGUUUACUAGUGCCAUU	95.2	88.3	93.8	93.9
458	21/181	30/191	345/509	355/519	GGUAUGUUGCCCGUUUG	94.6	96.1	98.1	80.2
382	24/184	37/199	348/512	363/527	UCUGCGGCGUUUUAUCA	96.7	95.6	95.3	95.8
1818	25/185	10/169	349/513	333/497	AACUUUUUCACCUCUGC	96.1	92.0	96.5	84.8
1576	26/186	26/187	350/514	351/515	CGUGUGCACUUCGCUUC	97.6	98.4	98.3	95.6
258	29/190	42/204	354/518	368/532	GGUGGACUUCUCUCAAU	91.9	83.6	97.3	90.5
189	31/192	91/254	356/520	418/582	UGCUCGUGUUACAGGCG	93.7	93.0	93.8	76.0
461	32/193	22/182	357/521	346/510	AUGUUGCCCGUUUGUCC	95.5	96.1	98.4	79.8
455	33/194	27/188	358/522	352/516	CAAGGUAUGUUGCCCGU	91.0	95.4	93.5	93.3
2317	34/195	34/196	359/523	360/524	CUUAUCAACACUUCCGG	89.8	89.8	94.8	77.7
1827	35/197	15/175	361/525	339/503	ACCUCUGCCUAAUCAUC	95.8	82.1	96.5	92.8
1655	36/198		362/526		UAAGAGGACUCUUGGAC	92.2	88.1	90.2	91.4
1438	38/200		364/528		GCGCUGAAUCCCGCGGA	95.5	94.1	92.0	79.6

FIG. 5

2419	39/201		365/529	GCGUCGCAGAAGAUCUC	94.6	89.6	92.3	83.2
1680	40/202		366/530	AUGUCAACGACCGACCU	96.7	83.9	93.6	93.5
671	41/203		367/531	CUCAGUUUACUAGUGCC	96.7	94.8	92.6	94.7
2414	43/205		369/533	UCGCCGCGUCGCAGAAG	88.6	84.1	95.1	92.4
686	44/206		370/534	CCAUUUGUUCAGUGGUU	96.7	90.4	96.0	97.5
1263	45/207		371/535	GAUCCAUACUGCGGAAC	97.3	89.9	93.8	95.6
1826	46/208		372/536	CACCUCUGCCUAAUCAU	96.1	81.8	96.5	91.6
260	47/209		373/537	UGGACUUCUCUCAAUUU	91.6	83.4	97.3	90.7
2821	48/210		374/538	GGUCACCAUAUUCUUGG	86.7	95.4	96.5	85.3
2417	49/211		375/539	CCGCGUCGCAGAAGAUC	94.9	94.8	95.0	92.2
2411	50/212		376/540	CAAUCGCCGCGUCGCAG	87.7	84.6	94.3	90.9
375	51/213		377/541	GGAUGUGUCUGCGGCGU	95.2	85.7	94.5	93.7
1859	52/214		378/542	ACUGUUCAAGCCUCCAA	68.4	91.9	97.0	96.4
676	53/215		379/543	UUUACUAGUGCCAUUUG	95.5	88.9	94.1	94.7
680	54/216		380/544	CUAGUGCCAUUUGUUCA	96.1	90.2	94.9	94.7
2418	55/217		381/545	CGCGUCGCAGAAGAUCU	95.2	94.8	95.0	92.4
2315	56/218		382/546	AUCUUAUCAACACUUCC	90.1	89.8	94.8	78.3
303	57/219		383/547	GCCAAAAUUCGCAGUCC	98.5	97.2	85.7	94.7
1825	58/220		384/548	UCACCUCUGCCUAAUCA	96.7	82.0	96.6	92.0
672	59/221		385/549	UCAGUUUACUAGUGCCA	94.9	88.5	93.8	94.1
463	60/222		386/550	GUUGCCCGUUUGUCCUC	94.9	95.1	98.4	78.9
682	61/223		387/551	AGUGCCAUUUGUUCAGU	96.1	89.9	94.8	94.5
1779	62/224		388/552	GGCUGUAGGCAUAAAUU	97.9	96.3	96.3	97.7
378	63/225	63/226	389/553 390/554	UGUGUCUGCGGCGUUUU	96.7	84.9	93.5	94.3
1584	64/227		391/555	CUUCGCUUCACCUCUGC	97.6	98.4	97.9	95.4
1577	65/228		392/556	GUGUGCACUUCGCUUCA	97.6	98.2	98.5	95.4
257	66/229		393/557	UGGUGGACUUCUCUCAA	91.9	83.6	97.1	90.5
379	67/230		394/558	GUGUCUGCGGCGUUUUA	96.1	84.9	93.6	94.1
457	68/231		395/559	AGGUAUGUUGCCCGUUU	91.3	94.8	96.7	79.2

FIG. 5

1684	69/232	396/560	CAACGACCGACCUUGAG	96.4	85.4	94.1	93.3	
1654	70/233	397/561	AUAAGAGGACUCUUGGA	92.2	87.8	90.2	91.2	
1683	71/234	398/562	UCAACGACCGACCUUGA	96.7	85.7	94.2	93.1	
2829	72/235	399/563	UAUUCUUGGGAACAAGA	87.0	96.7	97.1	85.1	
190	73/236	400/564	GCUCGUGUUACAGGCGG	94.9	93.3	94.0	76.0	
2412	74/237	401/565	AAUCGCCGCGUCGCAGA	88.0	85.7	95.1	92.8	
1860	75/238	402/566	CUGUUCAAGCCUCCAAG	68.4	91.7	97.0	96.4	
2416	76/239	403/567	GCCGCGUCGCAGAAGAU	88.3	83.6	93.8	90.7	
688	77/240	404/568	AUUUGUUCAGUGGUUCG	96.7	90.6	96.1	97.7	
1440	78/241	405/569	GCUGAAUCCCGCGGACG	95.8	95.3	92.8	79.8	
2820	79/242	406/570	GGGUCACCAUAUUCUUG	86.4	95.3	96.8	85.3	
1255	80/243	407/571	CCUCUGCCGAUCCAUAC	97.6	90.2	94.9	88.8	
1681	81/244	408/572	UGUCAACGACCGACCUU	96.7	85.7	94.3	93.7	
1829	82/245	409/573	CUCUGCCUAAUCAUCUC	95.8	82.8	97.0	89.3	
1575	83/246	410/574	CCGUGUGCACUUCGCUU	97.6	98.5	98.3	95.8	
1260	84/247	411/575	GCCGAUCCAUACUGCGG	97.0	88.6	92.9	95.2	
243	85/248	412/576	AGAGUCUAGACUCGUGG	93.7	96.9	95.7	96.0	
1861	86/249	413/577	UGUUCAAGCCUCCAAGC	68.4	91.7	96.9	96.4	
1777	87/250	414/578	GAGGCUGUAGGCAUAAA	97.9	95.8	96.3	97.7	
1776	88/251	415/579	GGAGGCUGUAGGCAUAA	96.7	95.4	96.2	97.7	
259	89/252	416/580	GUGGACUUCUCUCAAUU	91.9	83.7	97.5	90.7	
1817	90/253	417/581	CAACUUUUUCACCUCUG	95.8	91.7	96.2	84.4	
681	92/255	419/583	UAGUGCCAUUUGUUCAG	96.1	89.9	94.9	94.7	
1259	93/256	420/584	UGCCGAUCCAUACUGCG	96.7	88.5	92.8	94.9	
1578	94/257	421/585	UGUGCACUUCGCUUCAC	97.6	98.0	98.6	95.8	
191	95/258	422/586	CUCGUGUUACAGGCGGG	94.9	92.7	92.5	96.0	
2313	96/259	423/587	CUAUCUUAUCAACACUU	90.1	89.4	95.3	78.3	
2409	97/260	424/588	CUCAAUCGCCGCGUCGC	88.6	85.2	96.7	93.1	
1548	98/261	425/589	CCCGUCUGUGCCUUCUC	97.9	96.7	95.7	98.1	

FIG. 5

2314	99/262	426/590	UAUCUUAUCAACACUUC	90.1	89.8	94.9	78.3	
462	100/263	427/591	UGUUGCCCGUUUGUCCU	95.2	95.3	98.4	79.2	
460	101/264	428/592	UAUGUUGCCCGUUUGUC	95.5	96.1	98.3	79.6	
1585	102/265	429/593	UUCGCUUCACCUCUGCA	97.3	98.4	97.8	94.7	
1579	103/266	430/594	GUGCACUUCGCUUCACC	97.9	98.4	98.6	95.8	
304	104/267	431/595	CCAAAAUUCGCAGUCCC	98.5	97.4	85.9	95.2	
188	105/268	432/596	CUGCUCGUGUUACAGGC	93.7	93.0	93.8	75.8	
2267	106/269	433/597	GGAGUGUGGAUUCGCAC	93.7	96.4	94.4	97.3	
1686	107/270	434/598	ACGACCGACCUUGAGGC	96.4	85.9	93.8	93.1	
242	108/271	435/599	CAGAGUCUAGACUCGUG	93.1	96.7	94.9	92.6	
2413	109/272	436/600	AUCGCCGCGUCGCAGAA	88.6	84.1	95.0	92.8	
459	110/273	437/601	GUAUGUUGCCCGUUUGU	95.2	95.9	98.3	79.6	
1261	111/274	438/602	CCGAUCCAUACUGCGGA	97.3	89.8	94.1	96.0	
2311	112/275	439/603	CCCUAUCUUAUCAACAC	93.7	88.9	95.3	78.3	
675	113/276	440/604	GUUUACUAGUGCCAUUU	95.2	88.6	93.8	93.9	
1682	114/277	441/605	GUCAACGACCGACCUUG	97.0	85.7	94.4	93.1	
264	115/278	442/606	CUUCUCUCAAUUUUCUA	90.7	82.0	96.4	88.2	
1408	116/279	443/607	CGCGGGACGUCCUUUGU	95.5	96.9	95.9	94.5	
248	117/280	444/608	CUAGACUCGUGGUGGAC	95.5	97.2	96.5	97.1	
1264	118/281	445/609	AUCCAUACUGCGGAACU	97.9	89.4	94.1	95.6	
1257	119/282	446/610	UCUGCCGAUCCAUACUG	96.7	88.5	91.1	86.9	
1258	120/283	447/611	CUGCCGAUCCAUACUGC	96.7	92.5	92.2	88.4	
1256	121/284	448/612	CUCUGCCGAUCCAUACU	96.7	88.1	90.7	86.5	
1527	122/285	449/613	CACCUCUCUUUACGCGG	95.8	94.6	95.9	98.3	
2381	123/286	450/614	AGAACUCCCUCGCCUCG	91.6	95.8	97.3	89.9	
2383	124/287	451/615	AACUCCCUCGCCUCGCA	97.3	95.8	97.3	90.5	
267	125/288	452/616	CUCUCAAUUUUCUAGGG	90.1	82.3	96.4	87.6	
1523	126/289	453/617	GGCGCACCUCUCUUUAC	95.5	95.1	95.6	97.9	
1262	127/290	454/618	CGAUCCAUACUGCGGAA	97.6	89.9	94.0	95.8	

FIG. 5

2384	128/291	455/619	ACUCCCUCGCCUCGCAG	97.6	95.8	96.9	90.1	
1254	129/292	456/620	UCCUCUGCCGAUCCAUA	97.6	89.6	94.7	89.1	
2268	130/293	457/621	GAGUGUGGAUUCGCACU	93.7	93.8	93.5	97.3	
1522	131/294	458/622	GGGCGCACCUCUCUUUA	95.5	95.1	95.6	97.9	
2408	132/295	459/623	UCUCAAUCGCCGCGUCG	88.6	84.4	96.4	93.1	
1265	133/296	460/624	UCCAUACUGCGGAACUC	97.6	88.5	91.2	95.2	
1526	134/297	461/625	GCACCUCUCUUUACGCG	95.5	94.8	95.8	98.3	
1685	135/298	462/626	AACGACCGACCUUGAGG	96.4	85.2	94.1	93.3	
1267	136/299	463/627	CAUACUGCGGAACUCCU	97.6	88.1	90.1	95.2	
1443	137/300	464/628	GAAUCCCGCGGACGACC	95.5	95.4	92.3	79.2	
2382	138/301	465/629	GAACUCCCUCGCCUCGC	97.3	96.1	97.9	91.6	
1524	139/302	466/630	GCGCACCUCUCUUUACG	95.2	95.0	95.6	97.9	
1525	140/303	467/631	CGCACCUCUCUUUACGC	95.2	94.8	95.8	98.3	
1441	141/304	468/632	CUGAAUCCCGCGGACGA	95.8	95.3	94.1	79.6	
1583	142/305	469/633	ACUUCGCUUCACCUCUG	97.6	98.4	98.2	96.6	
2410	143/306	470/634	UCAAUCGCCGCGUCGCA	88.0	84.6	94.8	92.0	
1431	144/307	471/635	CCCGUCGGCGCUGAAUC	87.7	93.5	87.7	94.7	
1442	145/308	472/636	UGAAUCCCGCGGACGAC	95.8	95.4	92.3	78.9	
244	146/309	473/637	GAGUCUAGACUCGUGGU	93.7	96.7	96.0	95.8	
1266	147/310	474/638	CCAUACUGCGGAACUCC	97.6	88.3	89.9	95.2	
1439	148/311	475/639	CGCUGAAUCCCGCGGAC	95.8	94.8	92.1	79.8	
2270	149/312	476/640	GUGUGGAUUCGCACUCC	96.1	94.6	94.6	97.7	
187	150/313	477/641	CCUGCUCGUGUUACAGG	93.7	93.2	94.1	76.0	
1444	151/314	478/642	AAUCCCGCGGACGACCC	95.5	95.4	92.4	79.2	
1875	152/315	479/643	AGCUGUGCCUUGGGUGG	73.8	96.1	96.4	96.2	
186	153/316	480/644	CCCUGCUCGUGUUACAG	93.7	93.0	93.9	76.0	
1430	154/317	481/645	UCCCGUCGGCGCUGAAU	87.3	93.5	87.6	94.7	
2316	155/318	482/646	UCUUAUCAACACUUCCG	89.8	89.8	94.8	77.7	
2318	156/319	156/320 483/647 484/648	UUAUCAACACUUCCGGA	89.8	89.9	94.7	77.7	

FIG. 5

Table 6. NCBI Genbank accession Nos. of Hepatitis B Virus genomic sequences

Genotype A

FJ692613	FJ692587	AF090838	FJ692590	DQ020003	AF090839	GQ477476	GQ477473
FJ692584	EU859907	AJ131570	EU859910	FJ349223	FJ023662	AY862867	EU859928
AY233287	AY233279	FJ692609	AF297624	AB270536	GQ331048	AM295795	FJ692555
EU859904	FJ692610	FJ692563	GQ477496	EU859934	FJ692582	AB453982	EU594391
FJ692579	EU859927	EU859942	EU859930	GQ477492	AY233281	EU594394	FJ692588
AY738141	GQ477481	AY934765	AM184126	AF143305	EU859902	EU859951	AY934773
GQ477482	AY738142	AY161141	GQ331046	DQ788725	FM199974	FJ692570	FJ692575
FJ692559	AB453988	AY373428	EU859950	EU859914	EU859922	GQ331047	AY233276
EU859924	AM410963	GQ477498	FJ692571	EU859948	GQ477479	AF143301	EU859954
GQ414522	DQ298164	GQ477465	EU594395	FJ692569	GQ477484	FJ692607	EU859908
FJ904434	AJ131573	AF418674	AB453983	FJ692594	EF208113	AF143303	EU859898
FJ692565	AB241115	AM184125	GQ477477	DQ315784	EU859947	EU859931	FJ692556
EU859944	EF208115	EU185786	AB222707	EU054331	FJ692566	AF297625	GQ477470
EU859918	EU859941	FJ692572	AF043580	GQ477501	DQ298162	GQ477497	AY233288
DQ788729	FJ692560	EU859953	DQ315786	AB126580	GQ477460	AY903452	AY934770
FJ692598	AY934766	AY934774	FJ692596	FJ692603	U87746	EU859911	AY233275
AY934763	AB246317	AY077735	EU859916	EU859909	AB194952	FJ692591	FJ692576
DQ298161	GQ477466	AB453980	DQ788727	FJ692574	AF090841	FJ692606	AF043560
AB194951	FJ904411	AM295797	FJ692601	EU859955	AF143299	GQ477504	EU594392
EU859938	EU185788	FM199979	GQ477503	AY233277	AY233284	AF143300	AB453987
GQ477463	AY233282	GQ477489	AF143307	AY934772	GQ477480	FJ692583	EU859900
AF090842	FJ692581	GQ477474	EU859936	FJ692589	AY738143	AY233280	AY233283
EU859944	EU859901	AM282986	AF297622	EU594390	AM494718	GQ477464	FJ692580
EU859925	FM199977	AF143302	GQ477490	FJ692554	EU859926	GQ477499	AY738139
FJ692558	AM295799	FJ692593	AY902775	EU859929	AY128092	AY373429	EU185789
AY738140	GQ477487	AY233290	EU594393	GQ477472	FJ692611	AY934764	AB222708
GQ477483	EU859921	AY934768	EU859956	AB453986	AY233278	FJ692562	GQ477485
FJ692578	GQ477467	EU859913	AY233274	GQ477500	EU859906	EU859943	GQ477478
EU859905	GQ161813	FJ692604	FJ692577	FJ692602	AY233285	EU410082	EU859923
FJ692585	EF208114	FJ349224	AY934771	FJ692595	FJ692586	FJ692608	EU859903
AY233286	AY934767	EU859933	AY233289	EU859949	EU185787	AF143306	FJ349222
FJ692612	EU859940	GQ477468	GQ477471	FJ692568	AF418675	FJ692600	AB330371
GQ477462	FJ692561	GQ477495	EU859899	EU859915	GQ477494	GQ477502	AB330372
EU859925	AB241114	AM295800	FJ692557	DQ315785	GQ477469	DQ788726	AB330373
EU086721	DQ298165	GQ477475	FM199981	GQ184323	EU859932	EU859917	AJ627226
AB194950	AY034878	GQ477488	AB453985	AF143304	FJ692605	FJ692597	AJ627227
EU859939	EU859920	AM295796	EU366129	GQ477493	EU859912	EU747320	AJ627228
AF143308	GQ477486	AB453981	AF297623	AF297621	AY934769	FM199980	AP007263
FJ692599	FM199976	FJ692573	GQ477491	EU859935	FJ692592	AB453984	EU304331
DQ788728	AM295798	EU859952	EU859937	DQ020002	GQ184324	AB453979	EU414132
EU859919	FJ692567	EU414134	DQ298163	AF090840	EU859945	S50225	V00866
FJ692564	AB453989	FM199978	EU859946	GQ477461	AF143298		

Genotype B

EU306702	GU332692	AB073842	AY800389	AY206377	AB106884	AB073843	FJ386688
GU332701	AB073822	AB493832	DQ463798	FJ386636	EU939630	EU939633	AJ131574
EF473975	AY596102	GQ924634	EU939670	D23678	FJ386656	DQ463787	AB073840
FJ787444	DQ904357	AY167098	AY596103	DQ993680	AB116083	FJ386655	AB219429
D23679	EU882001	AB116082	AB073823	EF473974	GQ377641	AY293309	DQ361535
DQ993681	GQ924608	AB205122	GQ377596	AY781187	AF121243	U87747	AB493830
AY033072	GQ924654	EU306670	GQ377537	AY163870	GQ924635	EU564822	DQ993710
GQ924628	EU939671	EU939631	EU919175	EU306703	FJ386676	EU522074	AB365445

FIG. 6

EU919174	DQ463799	AB106885	GU332693	GU332700	AB493833	AB205120	EU882003
FJ562311	GQ377556	GQ924648	AY033073	FJ562222	FJ562262	FJ386675	FJ386582
FJ386615	GQ377568	EU305543	EU564823	GQ377549	FJ562312	AB073847	M54923
EU939673	DQ993683	AB219428	GQ377588	GU332702	GU332691	AB287317	EU660233
GQ924656	FJ386669	AB073841	AY217370	EU306701	EU939672	EU305545	AF121247
GU332690	GU332703	FJ032344	GQ924617	FJ386634	FJ386648	AP011087	GQ377573
EF103278	GQ377622	GQ924637	FJ386608	FJ386668	EU882002	FJ032342	EU522073
GQ377602	EU306700	AB205121	FJ386654	AY206375	DQ993711	GQ377639	FJ562246
EU919176	EU796068	AY330917	EU939632	DQ993682	FJ386583	GQ924611	EU564825
AB300364	EF473977	GQ377643	DQ448628	GQ377569	GQ924631	AY800391	X97851
GQ377595	FJ562260	EU522075	EF473976	AB073821	DQ993698	EU939634	DQ993684
AY220698	AB493831	FJ562240	FJ032358	GQ377594	AY217356	EU939668	AY206373
GQ377625	GU332697	GQ924610	AY217357	EU306695	EU306706	AB471854	AB493829
AF479684	EU919171	EU939669	DQ975271	EU939629	EU579441	AY217368	AB219430
GU332704	GQ377592	EU939635	DQ993699	EU939675	DQ993685	AY596105	EF473972
EU306707	AB073827	AY800390	GQ924630	EU881998	EU919173	GQ924653	DQ993686
AY781183	EU564824	AY163869	AB073826	FJ386584	EU306696	FJ386610	AF121245
EU882004	GQ377644	GQ377638	GU332696	FJ349296	GU332695	EU939676	AB073839
FJ349236	EU522072	EU305544	DQ377158	AY596106	FJ562316	EU306705	EU660231
EU939674	X97850	AB287316	GQ377604	EF473971	AB287329	GU332706	EU139543
EU939628	EU660232	AB073846	EU919170	AB100695	GQ377590	FJ562224	EU158262
GQ924651	AF121246	AP011086	GQ205440	GU332705	AB073825	AB205119	AB246335
EU330998	AB493835	GU332707	GQ377606	AB287314	EU660230	AY217364	AB073829
EU306677	AF282918	EU939677	GU332694	DQ993708	AY206380	AY596109	GQ377561
EU939636	AY206390	AY596104	AY217355	AY800392	FJ386660	EU881997	AB033555
DQ993709	EU487256	AY217369	AB241116	EU939637	EU306709	GQ924603	FJ386681
AY217374	AB241117	AB471855	GQ924632	EU158263	DQ995803	DQ463792	AY217358
EU796071	DQ993687	AB073824	EU487257	EU330999	FJ562289	FJ562254	DQ993696
AP011085	AB073858	AB287328	AY206391	FJ562219	D00329	GU332699	FJ518812
AB287315	EF473973	EU306697	AP011084	EU564826	AB073855	EU439022	AY167093
EU305547	EU306704	FJ562259	GQ377550	AB073838	AP011095	DQ980548	AP011089
AB073845	GQ377626	EU919172	AB073844	AF121244	DQ448620	AB287325	AB073849
AB287319	EU330994	AY217365	EU330995	DQ993697	GQ924641	FM209516	AB368295
FJ562234	EU570070	AB073854	AF121248	AY217359	EU939664	EU306698	GQ377629
EU939666	AB300371	DQ448621	AB073834	AB033554	EU939638	AB287327	AB493827
FJ386600	EU439023	AY220703	DQ993704	FJ386680	AY167102	EU439020	DQ995801
DQ993705	AB073828	AP011094	AY167101	GQ377582	DQ993707	AF100308	DQ448622
AY167100	AB287324	EU306708	GU357842	AB073837	AB010289	AY217366	FJ032352
AB073835	EU330989	DQ995802	EU939667	EU306679	FJ562236	AY766463	AB073857
AF121249	GU332698	AB300370	AP011088	EU919161	X98073	EU939678	GQ924621
AB231909	EU939627	EU919162	AB287318	EU306684	FJ386683	FJ386642	DQ993695
AB246339	DQ463793	EU570071	AB073848	EU330996	DQ993694	DQ463790	FJ518811
FJ386682	EU939639	DQ448623	EU306699	EF494381	EU939663	AB073850	AY217361
AF282917	EU939665	GQ377542	FJ562257	FJ386684	AY596110	D23677	DQ463797
X98072	EU330997	AB073856	GQ377558	DQ448619	AB195935	GQ924626	GQ924606
GQ377634	GQ377614	DQ463791	FJ032349	DQ463800	DQ993700	AB116090	AB212626
FJ562296	EU306678	EU939679	AB246340	AB073830	FJ787476	EU570069	AY596111
FJ562237	AB073836	AY217367	EU306710	EU570075	AB115551	AF121250	AB195934
EU305548	AY206383	AF100309	X98074	EU306683	AB117759	AB287320	FJ787477

FIG. 6

AB195933	AB302095	AY518556	D00330	GQ377612	AB010292	GQ377564	DQ993701
DQ993706	FJ032353	AB287326	FJ562231	FJ562303	AP011090	EU660224	GQ924647
GQ924640	AP011096	EU439021	GU168597	GQ924646	DQ448625	EU331000	FJ386658
EU939662	AB246341	GQ377565	AY217363	GQ924624	GQ924644	EU330993	EU589335
GQ377613	EF494380	AB287321	EU547563	AB010290	DQ993702	GQ924638	GQ377547
EU306682	GU168596	AF121251	DQ463795	DQ995804	EU350409	FM209513	AB073853
EU330990	D00331	EF134945	FJ562253	AB073852	AB212625	EU439019	EU796067
AB073831	X98075	GQ924627	EU522066	DQ448627	AY596112	AB219427	AP011093
DQ463801	DQ463796	DQ448624	GQ377566	FJ032357	GQ377587	GU168595	AY220704
GQ377525	GQ924607	FJ032354	AB287322	EU796066	AB073832	X98076	DQ448626
AY167097	AY217360	AP011091	EF134946	AP011092	DQ463802	FJ562321	AB010291
FJ562322	EU331001	AB073851	EU595030	DQ463789	GQ377610	EU306712	FJ386666
EU306711	GQ377539	GQ377519	AY206387	EU939661	EU306681	AB246342	AB486012
GQ924625	GQ924659	AB219426	DQ463788	AB493834	FJ023634	D50521	AB302943
EU595031	DQ463794	FM209512	GQ924645	AB493836	FJ023635	D50522	AB302944
AY167089	EU939681	GQ924639	AB014366	AF461360	FJ023636	FJ023631	AB302945
EU439024	AY217362	EU330992	AB031267	AJ627225	FJ023637	FJ023632	AB362933
EU660227	X98077	EU306680	AB302942	AY167094	FJ023638	FJ023633	AB493828
AY220697	AJ131133	AB073833	EU522067	AB246343	AF233236	GQ377567	GU168594
AB287323	EF494382	DQ993703	GQ475340	EU439018	EU939660	FJ787475	

Genotype C

FJ562331	EU439009	EU916218	FJ386580	FJ386617	FJ386677	GQ475351	AB250109
AY781186	D23684	EU570067	EU939547	EU439015	AB195947	AF537372	AB111117
FJ562282	FJ023664	AB300366	AY373432	FJ032347	EU939586	GQ377640	GQ924614
GQ377620	AF411411	GQ475311	FJ787464	EU305540	GQ227696	AY330914	DQ089764
GQ475331	EU939567	GQ377600	FJ787438	EU916224	FJ787458	EU919168	EU872003
FJ562223	FJ386637	EU306722	DQ089778	DQ377160	AB241110	FJ562243	AB026815
EU916238	AY206376	GQ377536	AF461043	EU560440	GQ377576	EU564820	FJ787478
GQ377516	EU939651	AB471853	DQ089785	EU589339	AB367417	EU916204	AF068756
FJ882612	DQ089758	GQ377597	EU871982	AF533983	EU589345	FJ386657	FJ787485
EF137802	EU306691	AB198077	AB112063	EU939611	AF223956	DQ089799	AY217373
EU678475	FJ787439	EU916219	EU939566	FJ562283	AY217372	FJ562242	DQ975274
AB367394	FJ787465	EU306690	FJ882613	GQ377621	DQ089765	EU919169	AB241111
EU871983	AB485810	EU306723	GQ377517	FJ787484	GQ924615	EU306671	AY167099
DQ089784	EU882000	FJ562310	EU439008	FJ787479	AB111116	AB205123	FJ787459
GQ924655	AB198076	EU939650	EF137803	AB026814	GQ924649	GQ475350	EU939610
GQ924609	AB471852	DQ089759	FJ562330	FJ023659	DQ089798	GQ377577	EU916225
DQ089779	GQ377601	GQ924629	DQ536412	EU872002	AF223957	AB367416	EU589338
FJ386616	GQ475310	AF411410	EU916239	AB112348	EU589344	GQ227697	EU560441
EU939546	AB300367	FJ787445	GQ475330	AB367395	EU916205	EU939587	EU410079
FJ386581	NC_003977	FJ023665	AB064314	EU678474	EU564821	AB195946	DQ377161
FJ032346	EU939559	EU306672	AB241112	DQ377162	EU522068	EU939565	GQ475333
EU305541	FJ787487	AY330916	EU939579	FJ562261	GQ475313	FJ023666	FJ562280
GQ377557	EU872001	GQ377642	GQ227694	EU916226	EU306693	FJ787446	AY781184
DQ089766	GQ259588	GQ475353	EU939584	FJ787466	AB471851	AB247916	FJ562333
AB111115	FJ032339	AF223954	AB195945	EU939545	GQ377534	AY206389	D16665
GQ924616	AB367415	AB116080	EU305542	FJ386649	AB367409	AB048704	FJ032359
FJ386609	GQ377574	FJ386629	GQ377554	EU871980	EU660228	EU939653	AB367429
AB195939	GQ377528	EU939613	AB367435	DQ089787	DQ993181	AY206374	GQ377548
AB367396	EU916206	GQ924636	FJ032345	EU306720	EU939598	FJ386635	EU554538
AY217371	FJ562241	AY161139	EU594383	FJ562313	EU939539	FJ562221	FJ882610
GQ377514	AY161138	GQ475352	GQ377575	AB367428	DQ536410	FJ787447	EU306692
DQ377163	AB195944	EU306673	EU939558	EU796069	FJ562332	FJ023667	AB300365

FIG. 6

DQ361534	EU939585	EU498227	AB367397	AB112408	AY781185	AF411412	GQ475312
EU916227	GQ227695	EU916207	AB195938	GQ377515	EU939652	GU385774	EU522069
AB300359	EU939578	EU589346	EU872000	EU554539	AB048705	GQ377535	GQ377603
GQ377555	EU939612	AB116081	FJ787486	FJ882611	AY206388	AB471850	FJ386614
EU439016	FJ386689	AF223955	AB111114	GQ377623	EU939564	AY220699	DQ089786
DQ478885	FJ386674	AB367414	DQ089767	FJ562281	EU787444	EU660229	EU871981
AB367434	FJ386628	FJ032338	AY152726	GQ475332	EU939538	AB367408	FJ787467
AB241113	AF537371	GQ377529	DQ478899	FJ562220	EU939599	EU306721	EU939544
AY206392	EU439010	GQ475328	FJ787480	AB246337	D23681	FJ787461	DQ089780
EU939648	AB367432	AB111112	AB367412	AF473543	FJ386632	AY596107	AY040627
EU939614	FJ562328	DQ089761	EF688062	AB116087	EU939608	FJ386578	EU871987
FJ386672	DQ377165	FJ386652	GQ475308	EU562219	EU939654	EU939542	FJ562314
EU414133	EU594384	FJ386599	GQ377619	FJ562308	GQ475334	FJ386585	DQ377159
AF411408	EU410080	AY217376	GQ475354	EU939562	FJ562287	AB037927	EU306727
EU939583	EU916221	EU678470	EU306675	AB176643	FJ562226	AB112066	EU306694
AB195942	FJ562266	AB111946	EU306688	AB241109	FJ562334	FJ386612	EU916241
GQ227693	EF494377	EU872006	EU589340	FJ023661	AB113878	DQ683578	GQ205441
GQ377553	AB493837	AB485808	EU560439	FJ787441	FJ882617	EU881999	AB300363
GQ475348	GQ377618	FJ386598	EU717218	FJ032343	AY206393	FJ386613	FJ882616
GQ377605	GQ475355	FJ787481	DQ377164	EU796072	GQ377593	EU871986	FJ562227
GQ475314	GQ475309	AB485809	FJ562329	EU439011	DQ980547	DQ089781	GQ377624
GQ377533	EU562218	AB026811	GQ475329	AF411409	EU554542	GQ924650	FJ562286
AP011108	EU560438	EU872007	EF494376	GQ227692	EU306726	FJ787460	GQ475335
AB112472	EU589341	DQ089760	FJ562267	AB195943	FJ562315	EU882005	FJ562335
EU306689	AB367413	AB111113	EU916220	EU939582	GQ475315	EU939543	AB074756
FJ562247	GQ377572	FJ386653	AB288026	FJ386673	GQ475349	FJ386579	AF462041
EU306674	EU678471	EU594385	GQ377552	EU939615	AB300362	AB113879	Y18858
EF536065	AY217377	EU410081	AB367433	EU939649	EU916240	AF384371	EU939655
EU939609	AB300361	DQ089782	AP011099	GQ377571	EF536066	EU872004	EU916223
AB202072	FJ562258	EU871978	D23683	AB471848	GQ475356	AB367393	EU594386
FJ386633	EU306725	AY707087	FJ023663	U87742	AB205125	EU678472	EU306719
AB176642	GQ377531	FJ562336	AY123424	FJ032360	EU522071	AB367430	GQ924633
EU939563	EU554541	AB074755	FJ787443	AB367410	FJ386650	FJ032340	FJ386670
D23680	FJ386587	FJ562278	EU939560	AB116078	DQ089763	EU439012	EU939616
FJ787440	EU939540	FJ562285	EU939656	EU589342	GQ924613	GQ377551	DQ975272
FJ023660	FJ787463	GQ475336	EU579442	FJ562244	EU871999	FJ562299	AB195940
GQ475316	EU882006	AF384372	AB202071	FJ562218	FJ787482	FJ562238	EU939581
GQ377607	EU871985	FJ882615	FJ386630	AB222714	AB026812	FJ562264	FJ386631
EU579443	AB205118	FJ787462	AB195941	EU306718	AY217375	AB116084	EU939591
EU939657	FJ562225	AB112471	DQ975273	EU594387	EU678473	EU522070	AB195950
FJ787442	FJ562279	GQ377530	EU939617	EU796070	AB367392	AB205124	EU939606
D23682	EU871979	EU554540	FJ386671	FJ032341	FJ386651	GQ475357	Y18857
EU939561	DQ089783	GQ377591	EU916222	AB367431	EU871998	AB222715	AB111120
FJ882614	EU871984	AB300360	AY057947	EU872005	GQ924612	FJ562245	GQ924623
AP011098	FJ386611	GQ475317	FJ562265	D28880	EU670263	AB471849	AF223961
FJ562337	AB112065	FJ562317	FJ562239	AB026813	DQ089762	GQ377570	FJ562228
GQ475337	EU939541	EU306724	FJ562298	FJ787483	AB116079	AB367411	FJ562274
FJ562284	FJ386586	EU939580	AB182589	AB115417	EU589343	FJ032361	EU916233
GQ377541	DQ089773	AP011106	EU155828	DQ922651	GQ872210	GQ377580	AB367401
DQ246215	AB368297	AB198081	AY641558	FJ562268	DQ089792	AB116089	AB198080
FJ032350	EU871974	FJ386620	DQ315783	EU717217	FJ904423	EU562217	GQ377560
AB299858	FJ386640	EU547558	GQ184325	EU306715	EU872008	FJ562306	DQ980549
AY220702	EU939626	EU939646	FJ562295	AB246345	FJ787473	FJ562248	AP011107
AB113876	GQ475346	FM209514	GQ377637	FJ562326	AB195930	EU306686	AY123041
AB367420	EU916213	EU939570	EF494379	AB074047	FJ386597	GQ475306	FJ562255
FJ386576	EU306729	FJ023673	AB493839	GU357843	AY217378	GQ377617	EU916212
EU872014	AB049609	FJ787453	GQ475326	GQ924643	EU939550	EU919163	AF286594
EU871989	AB367400	AJ309369	AJ344115	EU871995	GQ377521	DQ089804	GQ475347
EU306728	EU872015	FJ562275	AB246338	DQ890381	FJ349225	DQ922650	AY641559

FIG. 6

EU871975	GQ377540	FJ562229	FJ562307	GQ377581	AB195931	GQ475327	DQ315782
AB368296	FJ882618	FJ386661	AB231908	GQ377520	FJ386601	AB493838	FJ518813
EU881996	AB367421	Y18856	EU562216	FJ032331	GQ872211	EF494378	EU939571
DQ089772	AB113877	AY206381	AB116088	FJ787472	DQ089793	FJ562294	FM209515
EU871988	FJ032351	EU939607	AB493844	EU872009	EU871994	GQ377636	EU155829
FJ386641	AF223960	GQ924622	GQ377616	DQ986375	GQ924642	FJ562327	AY167092
AB037928	FJ562288	AB111121	GQ475307	AB365451	EU871969	AB246344	FJ787452
FJ386577	EU916232	AB195951	EU306687	EU939551	FJ562235	EU306714	FJ023672
AY596108	AY247032	EU939590	FJ562249	FJ386596	FJ562269	EU717216	EU939647
EU547559	FJ562304	FJ787471	GQ475324	FJ518810	DQ478900	EU881995	GQ377543
FJ386621	EU562215	DQ986376	GQ377635	EU939572	AP011104	DQ089771	DQ993688
FJ032332	D50489	AB365452	FJ562297	FJ562318	AB367402	AF498266	AB195952
GQ377523	DQ089790	DQ315781	EU939618	FJ562256	FJ787490	GQ475338	EU939593
AB206817	EU871997	AB042284	EU939644	EU916211	EU872016	EU916231	AB111122
AB300373	FJ386602	FJ562324	FJ386622	GQ475318	FJ386574	AY247031	AF458664
GQ377615	EU939552	EU594388	AB033557	GQ377609	AF182802	FJ562276	FJ386662
AB493847	FJ386595	EU306717	AY167091	GQ475344	FJ386589	AB367422	AY206382
EU570072	AB115418	EU717215	FJ023671	GQ377563	EU939624	AY220700	Y18855
AB116076	AB195932	AY057948	FJ787451	AB198083	EU871976	AP011097	EU939658
EU939604	EU306716	EU871996	AB206816	AB195953	GQ377628	AM180624	GQ475319
FJ787450	AB246346	DQ089791	GQ377522	DQ993689	FJ562339	FJ386575	EU916210
FJ023670	FJ562325	FJ386603	GQ377583	GQ372968	FJ386643	AP011105	EU717212
AY167090	AB042285	EU570073	GQ924620	AY220701	EU939625	DQ478901	EU589337
EU939573	GQ475325	EU306685	AB111123	AB113875	DQ089770	AB198082	FJ562323
FJ386623	GQ184326	GQ475305	AF458665	AB367423	EU871977	GQ377562	AB042283
EU939645	FJ386594	AB300372	EU939605	FJ562277	EU872017	AB367403	GQ475323
EU939619	EU939553	FJ562305	EU939659	AY247030	AY077736	FJ562319	GQ377632
AB033556	FJ787470	AB116077	FJ386663	EU916230	FJ386588	GQ377608	FJ562290
EU717214	AJ748098	FJ032333	EU939592	GQ475339	AF182803	GQ475345	FJ386679
FJ386625	AB367419	AB111119	EU439007	DQ089756	AP011103	EU939549	EU939554
EU939643	GQ377524	EU871990	AB367425	EU939603	GQ377599	AF182805	AB426467
AB033550	GQ377578	DQ089797	GQ377518	AY206385	AB198079	EU547561	DQ089796
FJ787456	GQ377585	FJ386605	GQ377544	FJ386639	AB198084	FJ386645	EU871991
AY167096	DQ980551	FJ386659	AB195955	FJ386665	GQ377538	FJ386619	AB111118
EU939575	AB493840	EU939555	EU939594	AY206378	AB367405	EU939623	FJ386604
DQ993693	EU919166	FJ386592	FJ349241	AB300368	AY641561	AY306136	EU919167
EU939588	AB298721	FJ562271	EU939569	GQ475343	DQ089800	DQ089776	AB493841
AB195949	AB049610	EU916236	AJ309370	EU916216	FJ787436	EU871971	EU570074
FJ032335	AF223958	FJ032355	AB111125	FJ562251	EU872011	FJ386593	AF223959
FJ562302	AF182804	FJ787468	FJ787489	EU939597	AB493843	AB014385	FJ023643
AB298720	EU939548	EU871973	EU939557	EU939536	GQ377611	AB014389	FJ023644
AB367418	AB198078	GQ924658	AB195937	AB367406	EU919165	AB014391	FJ023645
FJ032334	GQ377598	GQ924604	AB367398	AY641562	GQ377527	AB014392	FJ023646
GQ377584	AP011102	DQ089774	D12980	DQ089803	GQ377586	AB014393	FJ023647
DQ980550	DQ089801	DQ089789	GQ377526	AP011100	FJ032336	AB014394	FJ023648
GQ377579	EU660225	EU939621	FJ032337	EU916215	FJ787488	AB014396	FJ023649
FJ787457	AY641560	AB106895	FJ562301	FJ562252	AB367399	AB014399	FJ023650
AB195948	AB367404	FJ386647	EU919164	AY066028	AB195936	AB031262	FJ023653
EU939589	EU570068	EU916214	AF536524	FJ562340	EU939556	AB031265	FJ023654
DQ993692	FJ562250	GQ475341	AB493842	DQ089788	FJ386591	AB076678	FJ023656
EU939574	EU916217	DQ089802	EU916208	DQ089775	FJ386606	AB076679	FJ023657
EU939642	GQ475342	EU660226	AB033552	EU871972	GU357845	AB105172	FJ023658
FJ386685	AB300369	EU439025	EU939641	FJ386646	GQ924619	AB105173	FJ023668
FJ386624	DQ089757	AY641563	FJ386686	EU547562	DQ089769	AB105174	FJ023674
FJ386678	AB111124	AB367407	FJ386627	EU939620	EU871993	AB116085	FJ023675
AB033551	AY206379	AP011101	DQ993691	FJ787469	DQ089794	AB362931	FJ023676
AB042282	FJ386664	FJ787448	EU939577	EU872012	AB014360	AB362932	L08805
EU589336	FJ386638	EU939537	FJ787454	GQ475320	AB014362	AB367800	L13994
M38636	AY206384	EU939596	FJ562233	FJ562293	AB014363	AB367803	M38454

FIG. 6

EU717213	EU939602	AB195957	EU916228	GQ377631	AB014364	AB367804	S75184
FJ562230	EU939568	FJ386667	FJ562292	EU916229	AB014365	AF461357	V00867
GQ377633	EU939595	EU939601	GQ377630	FJ562232	AB014367	AF461358	X01587
FJ562291	AB195954	AF363962	GQ475321	EU717211	AB014369	AF461359	X02763
GQ475322	AJ309371	EU916234	AB205152	EU306713	AB014370	AF461361	X04615
AB050018	AB367424	FJ562273	EU554537	FJ562320	AB014371	AF461363	X14193
GQ377559	EU439006	EU554536	FJ032356	EU939576	AB014372	AJ012207	X52939
FJ032348	EU554535	GQ377546	AF241410	AF330110	AB014374	D00630	X70185
EU939622	GQ377545	AB367427	AB367426	DQ993690	AB014376	D16666	X75656
FJ386618	AB117758	AF241411	AF363963	FJ787455	AB014377	D16667	X75665
EU547560	DQ922649	EU439005	FJ562272	AY167095	AB014378	D50517	Z35717
FJ386644	EU916237	FJ386607	EU916235	AB033553	AB014379	D50518	Z72478
EU871970	FJ562270	DQ089795	EU939600	FJ386626	AB014380	D50519	Z72479
DQ089777	AF363961	EU871992	AY206386	FJ386687	AB014381	D50520	FJ023642
EU872010	AY148342	DQ089768	FJ787449	EU939640	AB014382	FJ023639	AB014384
FJ787437	X51970	GQ924618	FJ023669	FJ562300	AB014383	FJ023641	EU916209
AM180623	EU872013	FJ787474	AB195956				

Genotype D

AY721606	AF121240	GU456638	FJ349214	EU594409	EU594389	GU456672	AB330369
FJ904397	GQ377589	AY161162	EU787440	FJ904403	AJ344116	M32138	AB330370
FJ904414	EU919197	AB270543	GQ167302	AY741797	AY721612	FJ904447	AF280817
EU787447	AB222711	AY236163	AB048701	AY721611	DQ315780	FJ904398	AJ627215
FJ349213	EU594400	GU456644	DQ111987	EU414141	AY741794	AY721609	AJ627216
GU456680	GU456663	GQ205382	EU594425	AY902770	AY233293	X59795	AJ627217
X97848	EU921418	EU594427	GQ205380	AB119256	FJ904420	EU787437	AJ627218
AJ131956	DQ315777	DQ486024	GU456646	AB493845	EU414142	FJ904438	AJ627219
FJ349233	L27106	AB188245	FJ349228	X80925	AY902773	AY161157	AJ627220
AB270546	AB270544	AB205127	AB270541	AY862864	GU456649	GU456651	AJ627221
FJ562263	AY236164	EU787442	AF121239	GU456657	AB119255	AB090269	AJ627222
EU414136	FJ904436	FJ349216	AY236161	EU594434	AB493846	EU594432	AJ627223
GU456661	AY161159	AF418687	GU456666	AY161151	GU456675	EU939680	AJ627224
AB210821	AY373430	GU456678	AB033559	AB270550	EU594416	EF103276	DQ336674
EU594402	EU787439	AB048703	EU594405	EU594397	FJ904440	AY796030	DQ336675
FJ904408	FJ349231	EU594398	FJ349208	GQ477455	GQ922000	AM494716	DQ336676
AF121242	EF103285	FJ904431	AY161160	GQ922002	GQ477457	FJ904418	DQ336677
DQ304548	AY661793	GU456658	DQ111986	GU456677	EU594436	FJ904444	DQ336678
AB222713	DQ329357	AB210818	AF418684	FJ904442	GU456655	GU456671	DQ336679
EU594422	FJ349211	AB471856	FJ904412	AF418688	AY161153	GQ477453	DQ336680
AY945307	EU787445	DQ304551	AF418679	FJ349219	AB109476	AY902777	DQ336681
GU456641	FJ904416	AB205126	EU787441	AB205128	AY233295	FJ904424	DQ336682
GQ205387	AF418680	FJ562309	FJ349215	FJ904422	AB270548	FJ386590	DQ336683
FJ904428	GU456682	DQ486025	AF043594	EU594428	EU414138	AB119251	DQ336684
AY161147	FJ904395	AB188244	GQ477459	EU414140	FJ904406	AY090453	DQ336685
DQ486021	EU594382	GQ205383	GQ924652	AY721610	AF418690	FJ904404	DQ336686
Y07587	DQ315776	GU456645	FJ349235	AY233291	AB119253	AF418692	DQ336687
FJ349232	AB270545	EU594426	FJ904432	AY741796	GQ205389	DQ315779	DQ336688
FJ904435	EU921419	AY236162	AB109478	FJ349205	GU357846	GQ184322	DQ336689
X97849	EU594401	AY741798	AB110075	FJ904402	FJ349221	GQ477452	DQ336690
GU456681	EU414135	AB270542	AB222709	GU456637	FJ904426	GQ922005	DQ336692
FJ904415	GU456662	AY161163	EF103281	EU594408	AY161149	GU456670	DQ464164
FJ349212	AB210822	AB267090	FJ349209	AB119254	AY721608	FJ904445	DQ464165
EU787446	AB222710	EU594406	AY161161	GU456648	FJ904399	EU155893	DQ464166
AB116266	AB270539	GU456639	AB033558	AY902772	FJ904446	FJ904419	DQ464167
FJ904396	AF121241	GU456665	GU456667	EU414143	GU456673	DQ991753	DQ464168
AF043593	AB078033	AB471857	EU594404	AB090270	EU594410	AY796031	DQ464169
AY721607	DQ486022	GQ377532	AY236160	FJ904421	AY796032	EF103277	DQ464170
AB188241	AB188243	DQ304550	AB246348	AY741795	AY161155	GU456650	DQ464172

FIG. 6

FJ904429	GQ205379	AB210819	AB270540	AY233292	EU594430	EU594433	DQ464173
AB078031	GU456642	GU456659	FJ349229	AJ132335	GU456653	AB090268	DQ464174
EU594423	GQ205384	FJ904430	EU594424	AB246347	AY902769	EU787436	DQ464175
GQ205386	EU594421	EU594399	GU456647	AJ344117	FJ349220	AY161156	DQ464178
GU456640	FJ904394	GU456684	GQ205381	DQ399006	AY161148	FJ904439	DQ464181
AY341335	AY721605	GU456679	AB493848	GU456668	FJ904427	AB126581	DQ464182
AB222712	GU456683	GQ167301	AF418689	FJ904401	AY902774	AY233296	GQ922004
DQ304549	FJ349210	AB048702	FJ904443	FJ349206	GQ205388	DQ315778	X02496
FJ904409	AF418681	FJ349217	FJ349218	AB109477	AB119252	FJ904405	X65257
AB210820	FJ904417	EU787443	EU594415	X80926	DQ304547	AY090452	X65258
GU456660	EF103279	AF418686	GU456676	AY161152	AF418691	AB270537	X65259
EU414137	AY661792	FJ904410	GQ477454	GU456654	FJ904407	FJ904425	X68292
EU594403	DQ329356	EF103280	EU594396	GQ922001	EU414139	AY902776	X72702
AB270547	AY373431	AB109479	GQ922003	FJ562338	AB270549	GQ205377	X85254
GQ205385	AY161158	AM422939	AY161150	GQ477456	U95551	AB104709	Z35716
GU456643	FJ904437	FJ349234	GU456656	FJ904441	AY233294	AB104710	V01460
GQ205378	FJ349230	FJ904433	EU594435	GU456674	EU594431	AB104711	AB330368
DQ486023	EU787438	GQ477458	AF151735	FJ904400	AY902768	AB104712	EF103275
AB078032	EU594407	GQ377627	X80924	FJ349207	GU456652	AB330366	GU456635
AB188242	AB120308	FJ904413	AB109475	GU456669	AY161154	AB330367	GU456636
AB270538	GU456664	AF418685					

FIG. 6

Table 7.Comparision of knockdown efficacies and coverage of HBV genomes for single dsRNAs and combinations thereof.

dsRNA 1				dsRNA2				Combination of dsRNA 1+2				
SEQ ID NO pair	1 nM		% coverage (of 2754 genomes)	SEQ ID NO pair	1 nM		% coverage (of 2754 genomes)	10 nM	1 nM		% coverage (of 2754 genomes)	Genomes not matched
	[%] rem. Rluc	rank			[%] rem. Rluc	rank		[%] rem. Rluc	[%] rem. Rluc	rank		
322/486	14	1	96.4	333/497	21	7	93.5	5	25	1	99.67	9
322/486	14	1	96.4	346/510	35	13	94.3	7	26	2	99.82	5
322/486	14	1	96.4	330/494	20	5	92.2	6	28	3	99.67	9
322/486	14	1	96.4	324/488	15	2	95.8	5	29	4	99.85	4
327/491	19	4	92.6	322/486	14	1	96.4	5	30	5	99.64	10
327/491	19	4	92.6	326/490	17	3	93.3	4	30	6	99.35	18
326/490	17	3	93.3	333/497	21	7	93.5	4	30	7	99.71	8
336/500	23	8	90.2	322/486	14	1	96.4	5	31	8	99.64	10
324/488	15	2	95.8	333/497	21	7	93.5	3	31	9	99.56	12
324/488	15	2	95.8	339/503	25	10	92.6	5	31	10	99.75	7
326/490	17	3	93.3	347/511	36	14	96.5	6	31	11	99.82	5
326/490	17	3	93.3	322/486	14	1	96.4	5	32	12	99.85	4
332/496	21	6	94.0	322/486	14	1	96.4	6	32	13	99.85	4
332/496	21	6	94.0	324/488	15	2	95.8	4	32	14	99.31	19
327/491	19	4	92.6	332/496	21	6	94.0	4	32	15	99.38	17
332/496	21	6	94.0	347/511	36	14	96.5	5	32	16	99.89	3
327/491	19	4	92.6	324/488	15	2	95.8	4	33	17	99.78	6
336/500	23	8	90.2	324/488	15	2	95.8	5	33	18	99.49	14
332/496	21	6	94.0	333/497	21	7	93.5	3	34	19	99.71	8
324/488	15	2	95.8	347/511	36	14	96.5	5	34	20	99.85	4
332/496	21	6	94.0	330/494	20	5	92.2	4	37	21	99.24	21
337/501	24	9	95.2	322/486	14	1	96.4	6	42	22	99.82	5
337/501	24	9	95.2	347/511	36	14	96.5	6	42	23	99.89	3

FIG. 7

337/501	24	9	95.2	324/488	15	2	95.8	5	43	24	99.60	11
337/501	24	9	95.2	333/497	21	7	93.5	6	44	25	99.71	8
337/501	24	9	95.2	336/500	23	8	90.2	7	47	26	99.71	8
341/505	31	11	91.5	322/486	14	1	96.4	5	50	27	99.85	4
341/505	31	11	91.5	324/488	15	2	95.8	5	57	28	99.67	9
351/515	38	15	97.7	337/501	24	9	95.2	6	60	29	99.75	7
351/515	38	15	97.7	342/506	32	12	93.2	8	60	30	99.93	2

FIG. 7

Table 8. Sequences of the negative control ds RNAs used in the psiCHECK™-2 screening assay.

strand	Sequence	gene
sense	5′-cuuAcGcuGAGuAcuucGATsT-3′	LUC(GL3)
antisense	5′-UCGAAGuACUcAGCGuAAGTsT-3′	LUC(GL3)
sense	5′-CcAcAuGAAGcAGcACGACusU-3′	GFP
antisense	5′-AAGUCGUGCUGCUUCAUGUGgsusC -3′	GFP

FIG. 8