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(54) **RNA INTERFERENCE MEDIATED INHIBITION OF HIV GENE EXPRESSION USING SHORT INTERFERING RNA**

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(60) Provisional application No. 60/398,036, filed on Jul. 23, 2002. Provisional application No. 60/294,140, filed on May 29, 2001.

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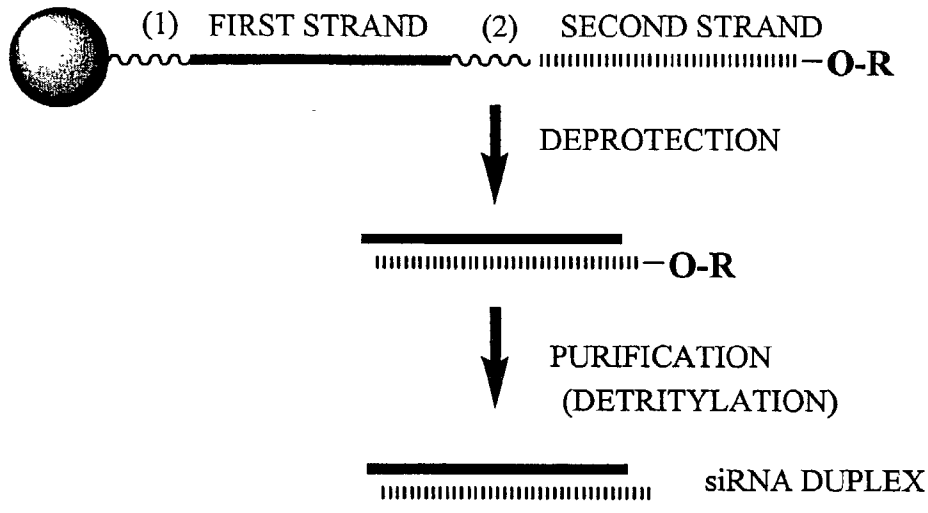
(57) **ABSTRACT**

The present invention concerns methods and reagents useful in modulating HIV gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to small interfering RNA (siRNA) molecules capable of mediating RNA interference (RNAi) against HIV polypeptide and polynucleotide targets.

Figure 1

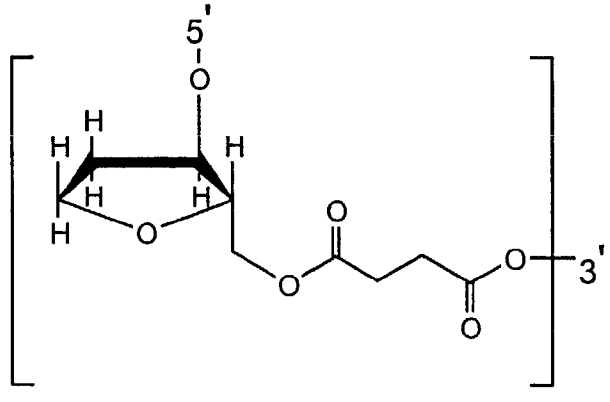


= SOLID SUPPORT

R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE

Figure 2

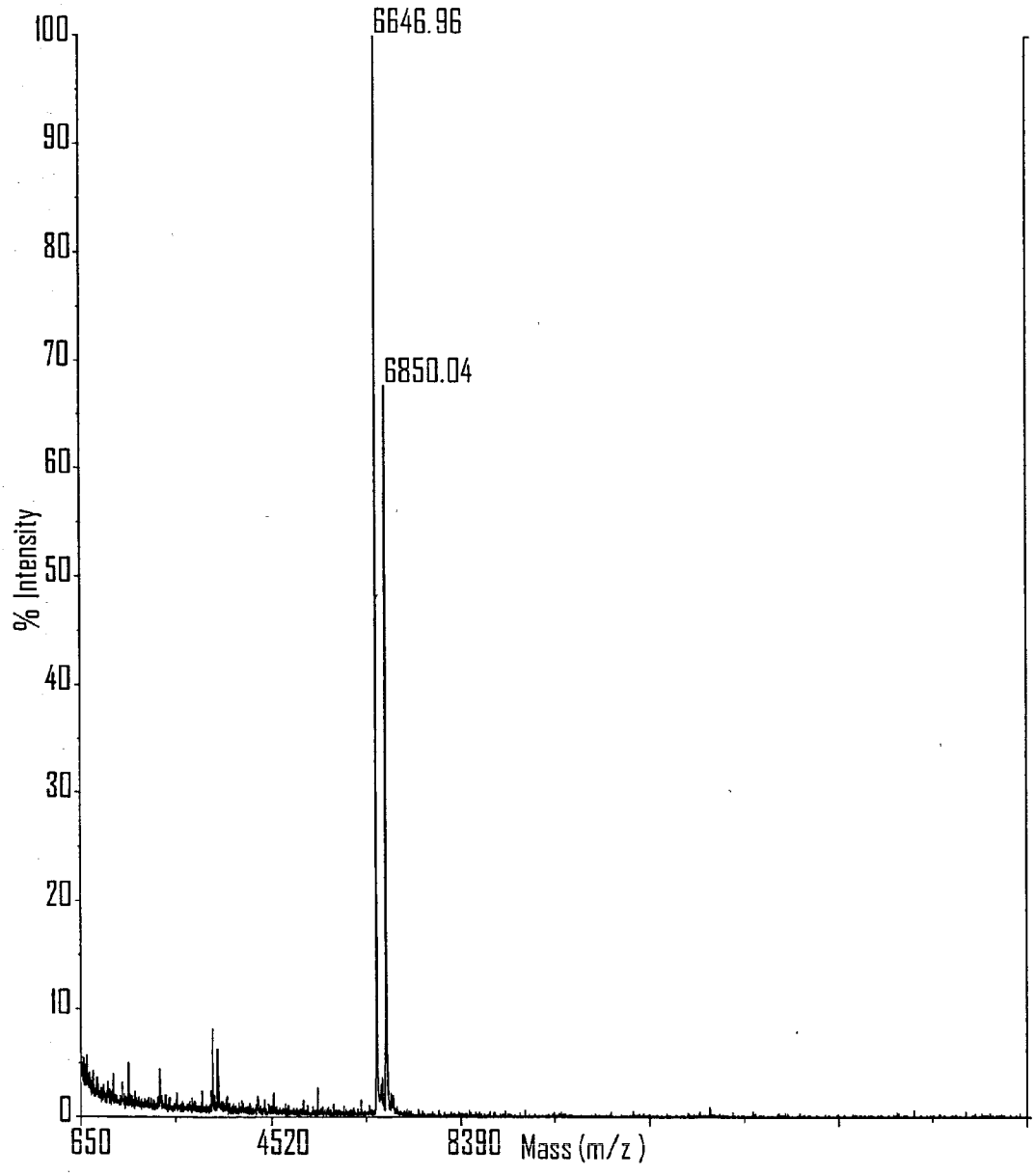

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

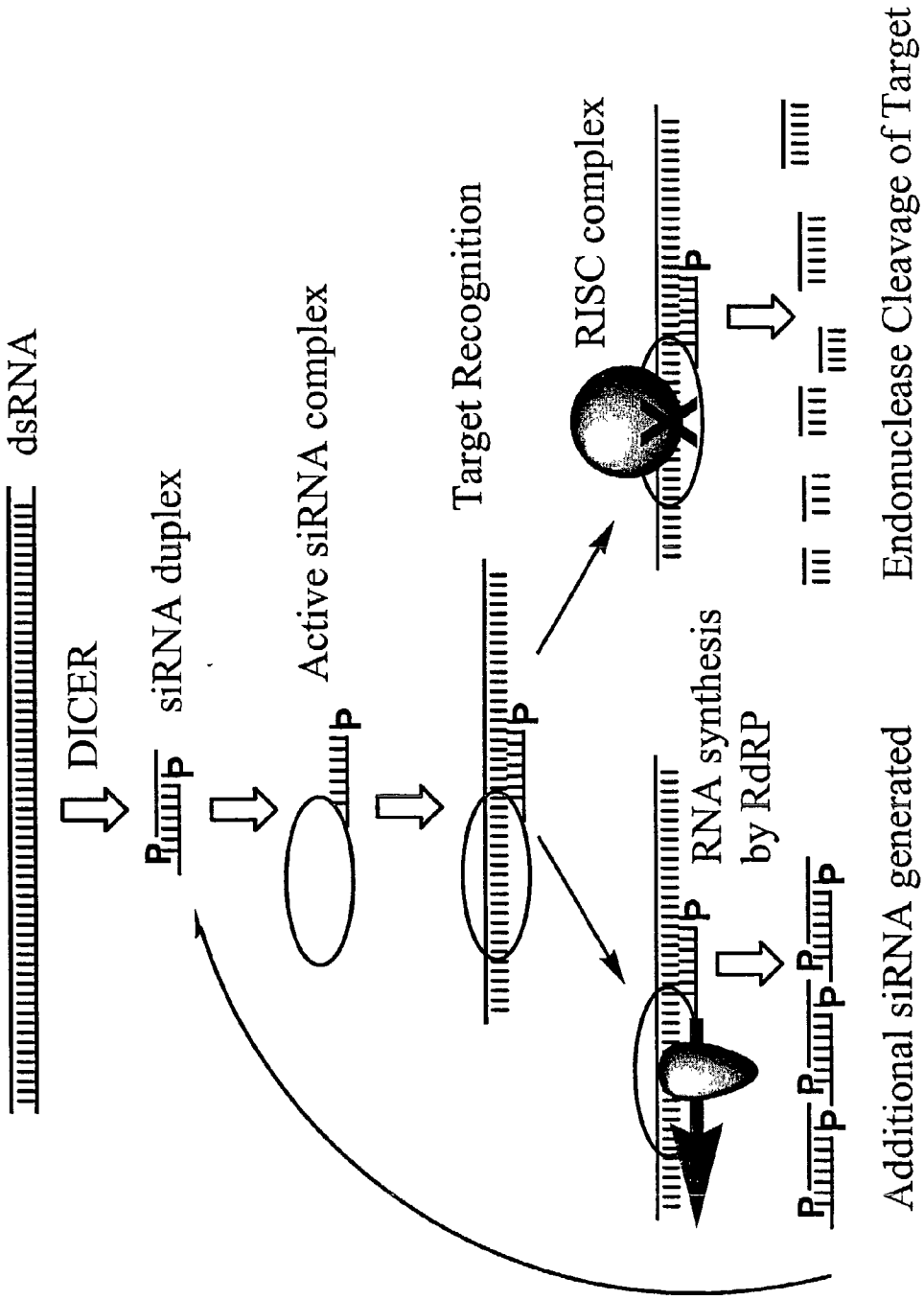
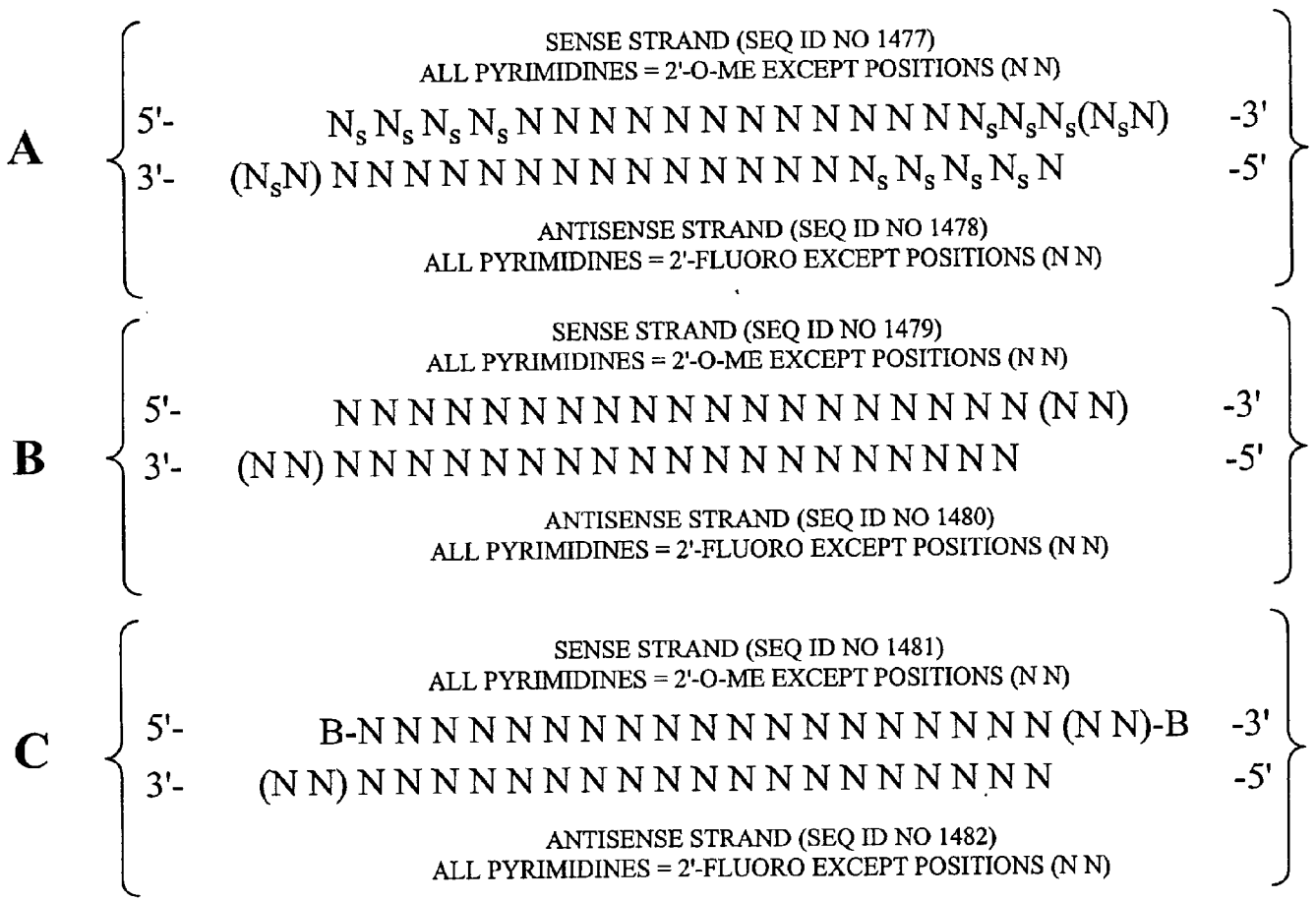
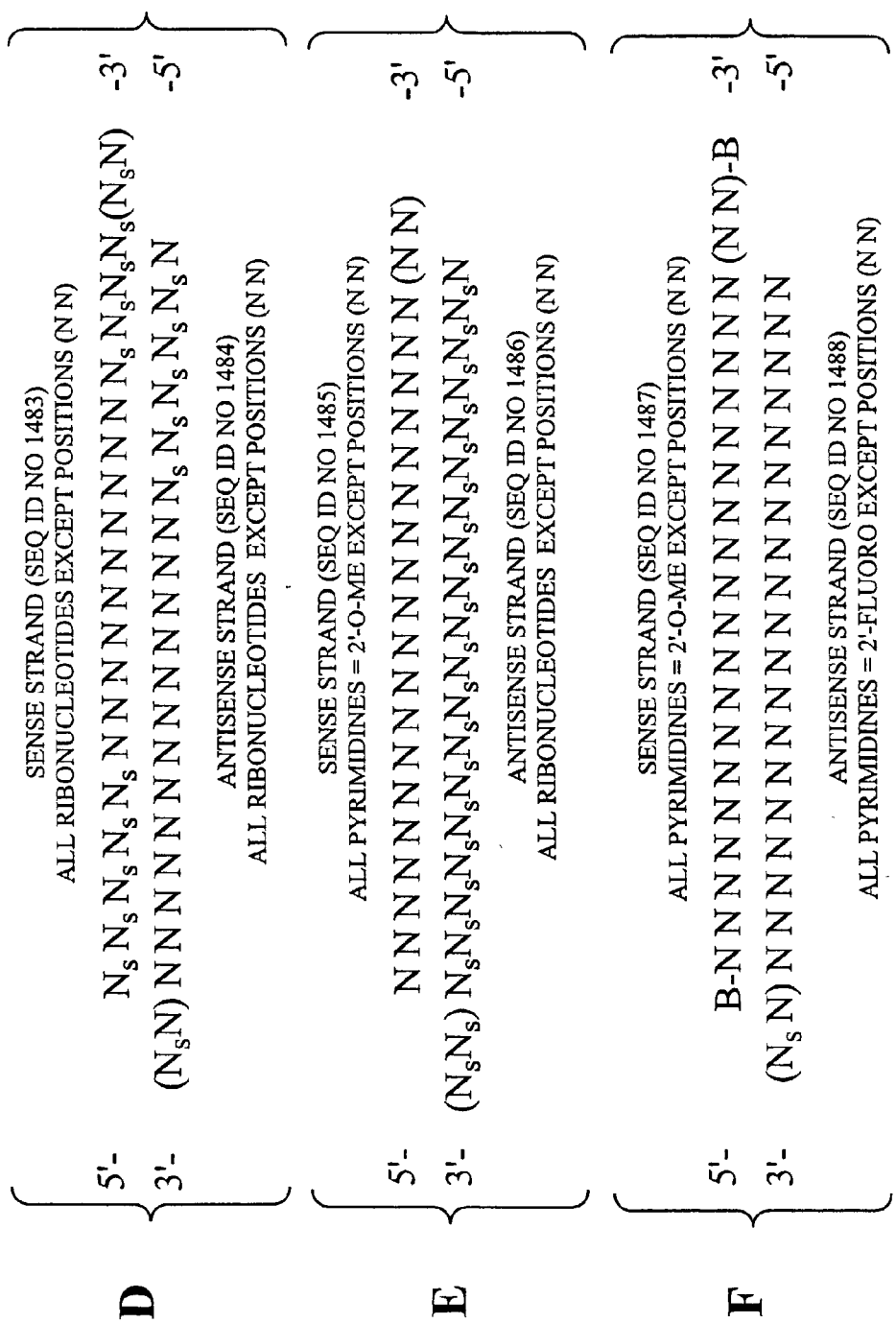

dsRNA
DICER
siRNA duplex
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis
by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated
by DICER

Figure 4



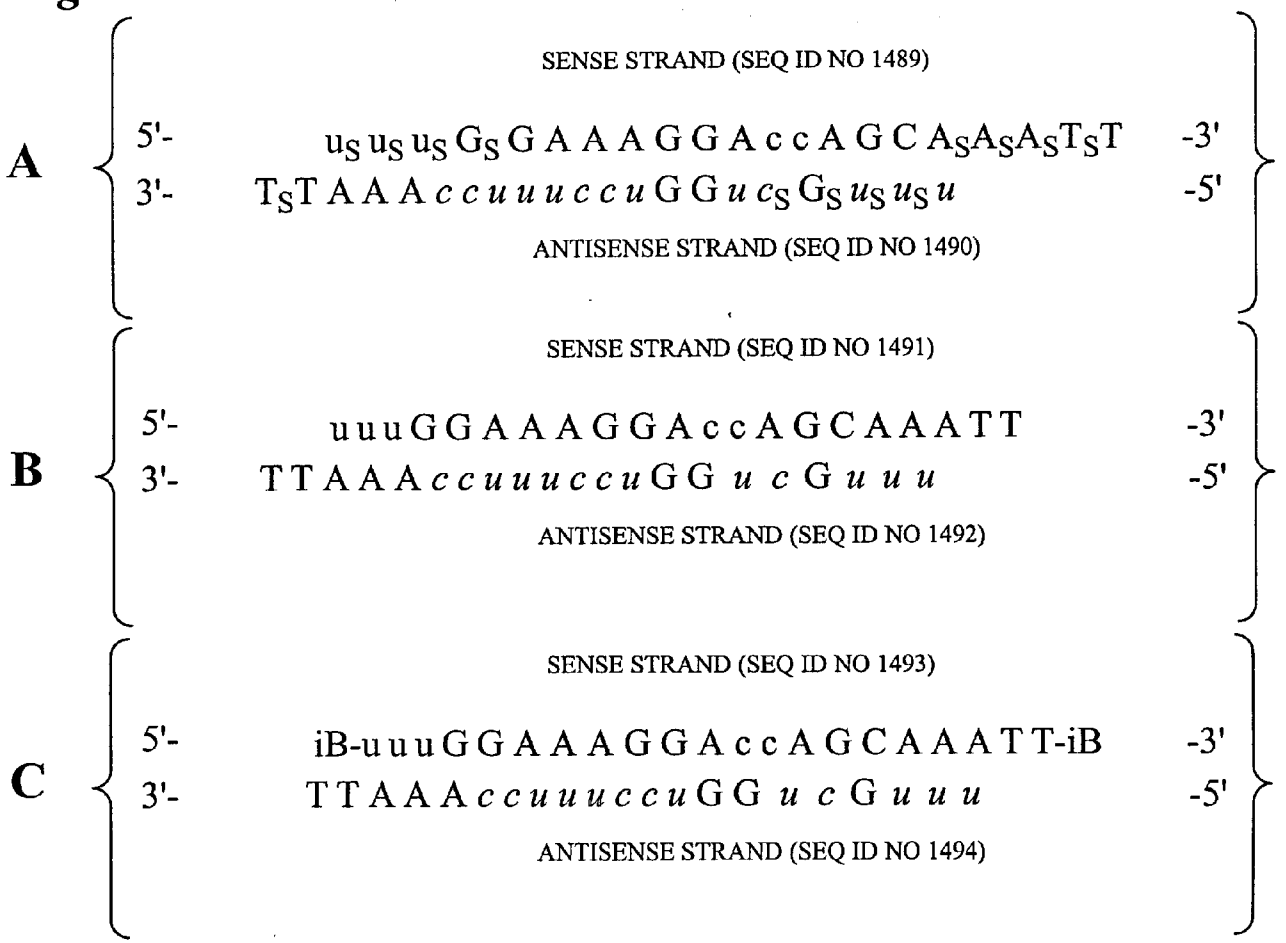
POSITIONS (NN) CAN COMPRISE UNIVERSAL BASE OR DEOXY (eg. THYMIDINE)
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 4



POSITIONS (NN) CAN COMPRISE UNIVERSAL BASE OR DEOXY (eg. THYMIDINE)
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 5



lower case = 2'-O-Methyl; *italic lower case = 2'-deoxy-2'-fluoro*
iB = INVERTED DEOXYABASIC
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 5

D

SENSE STRAND (SEQ ID NO 1495)

5'- $U_SU_SU_SG_SG_S$ A A A G G A C C A G $C_SA_SA_SA_ST_ST$ -3'

3'- T_ST A A A C C C U U C C U G G U_S C_S G_S U_S U_S U -5'

ANTISENSE STRAND (SEQ ID NO 1496)

E

SENSE STRAND (SEQ ID NO 1497)

5'- u u u G G A A A G G A c c A G C A A A T T -3'

3'- $T_ST_SA_SA_SA_SC_SC_SC_SU_SU_SC_SC_SU_SG_SG_SU_S$ C_S G_S U_S U_S U-5'

ANTISENSE STRAND (SEQ ID NO 1498)

F

SENSE STRAND (SEQ ID NO 1499)

5'- iB-u u u G G A A A G G A c c A G C A A A T T-iB -3'

3'- T_S T A A A *c c u u u c c u* G G *u c* G *u u u* -5'

ANTISENSE STRAND (SEQ ID NO 1500)

lower case = 2'-O-Methyl; *italic lower case = 2'-deoxy-2'-fluoro*
iB = INVERTED DEOXYABASIC
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE

Figure 6

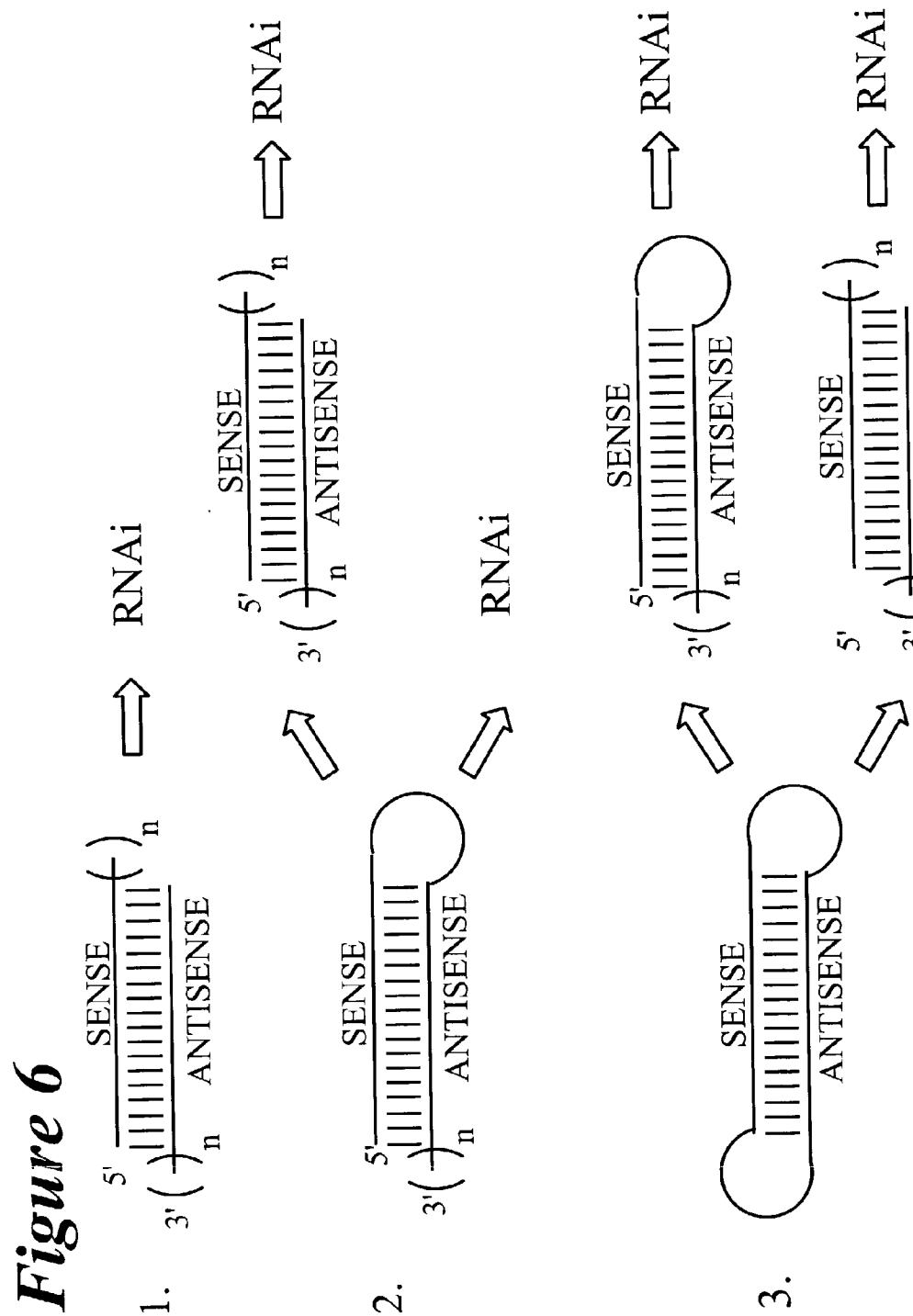


Figure 7

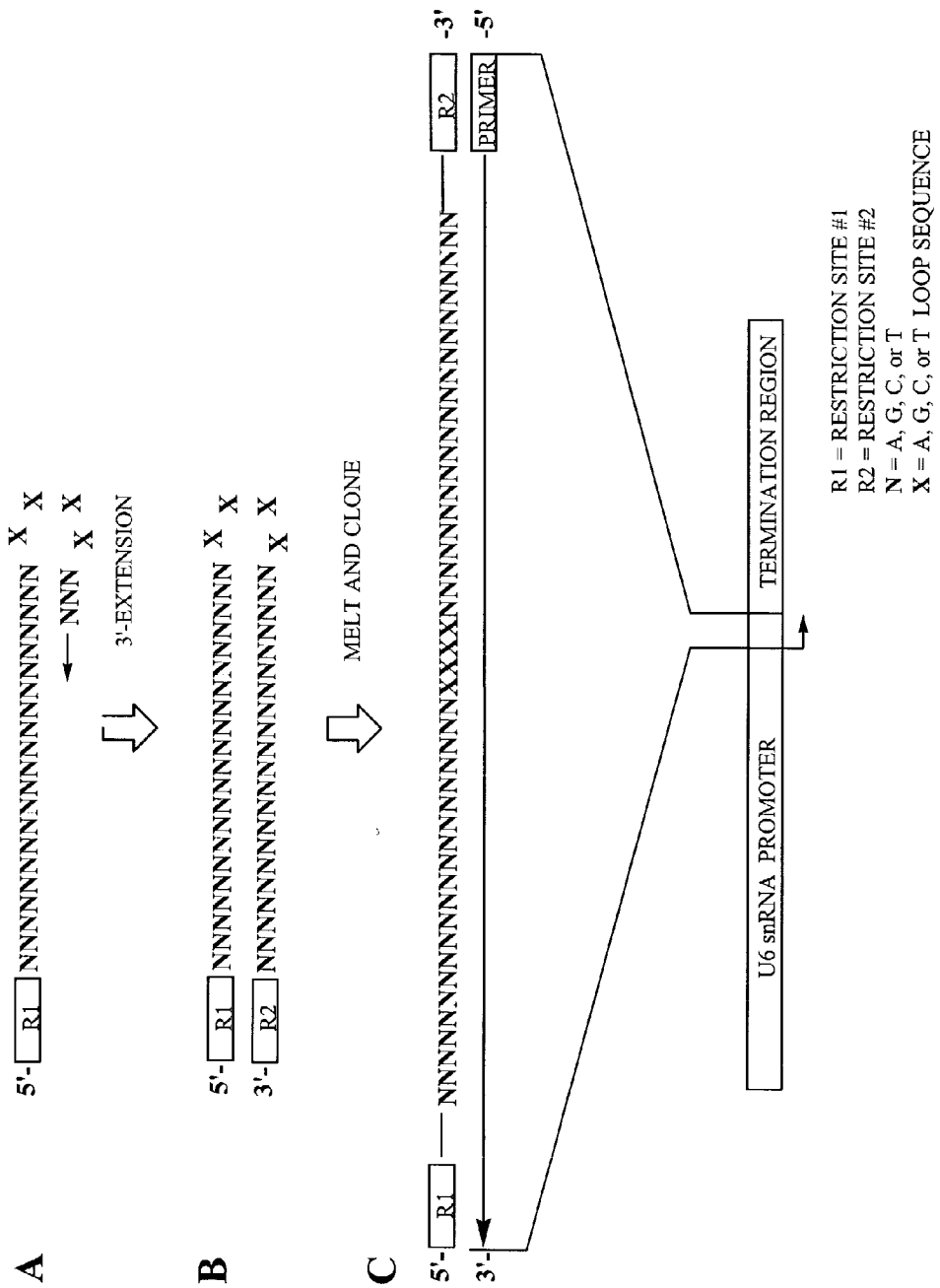
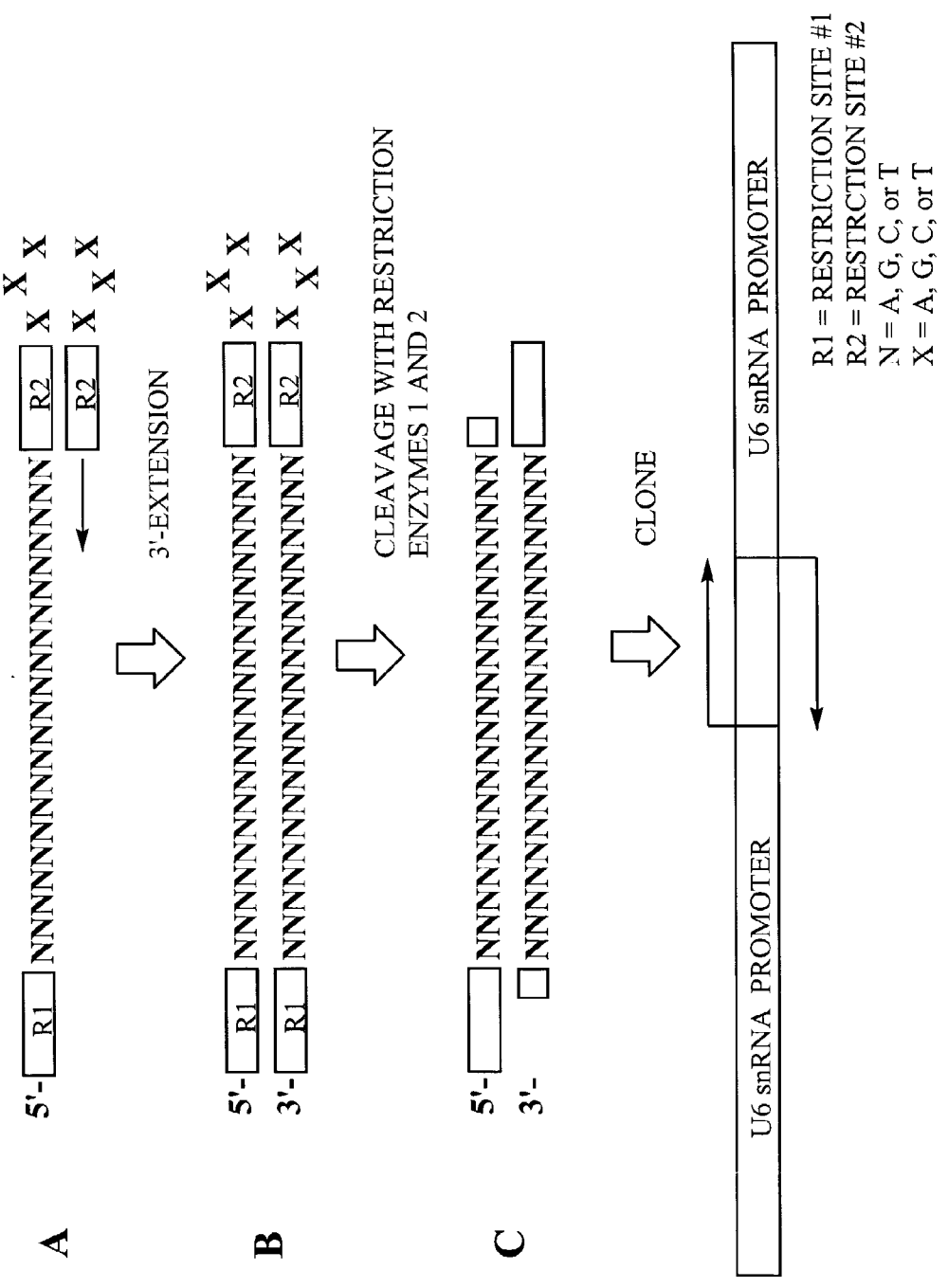

A
5'- R1 NNNNNNNNNNNNNNNNNNN X X
NNN X X
3'-EXTENSION
B
5'- R1 NNNNNNNNNNNNNNNNNNN X X
3'- R2 NNNNNNNNNNNNNNNNNNN X X
MELT AND CLONE
C
5'- R1 — NNNNNNNNNNNNNNNNNNNNNNXXXXNNNNNNNNNNNNNNNNNNN — R2 -3'
3'- PRIMER -5'
U6 snRNA PROMOTER
TERMINATION REGION
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T LOOP SEQUENCE

Figure 8


A
5'-
R1
NNNNNNNNNNNNNNNNNNNN
R2
X X X
X X X
3'-EXTENSION
B
5'-
R1
NNNNNNNNNNNNNNNNNNNN
R2
X X X
3'-
R1
NNNNNNNNNNNNNNNNNNNN
R2
X X X
CLEAVAGE WITH RESTRICTION ENZYMES 1 AND 2
C
5'-
NNNNNNNNNNNNNNNNNNNN
3'-
NNNNNNNNNNNNNNNNNNNN
CLONE
U6 snRNA PROMOTER
U6 snRNA PROMOTER
R1 = RESTRICTION SITE #1
R2 = RESTRICTION SITE #2
N = A, G, C, or T
X = A, G, C, or T

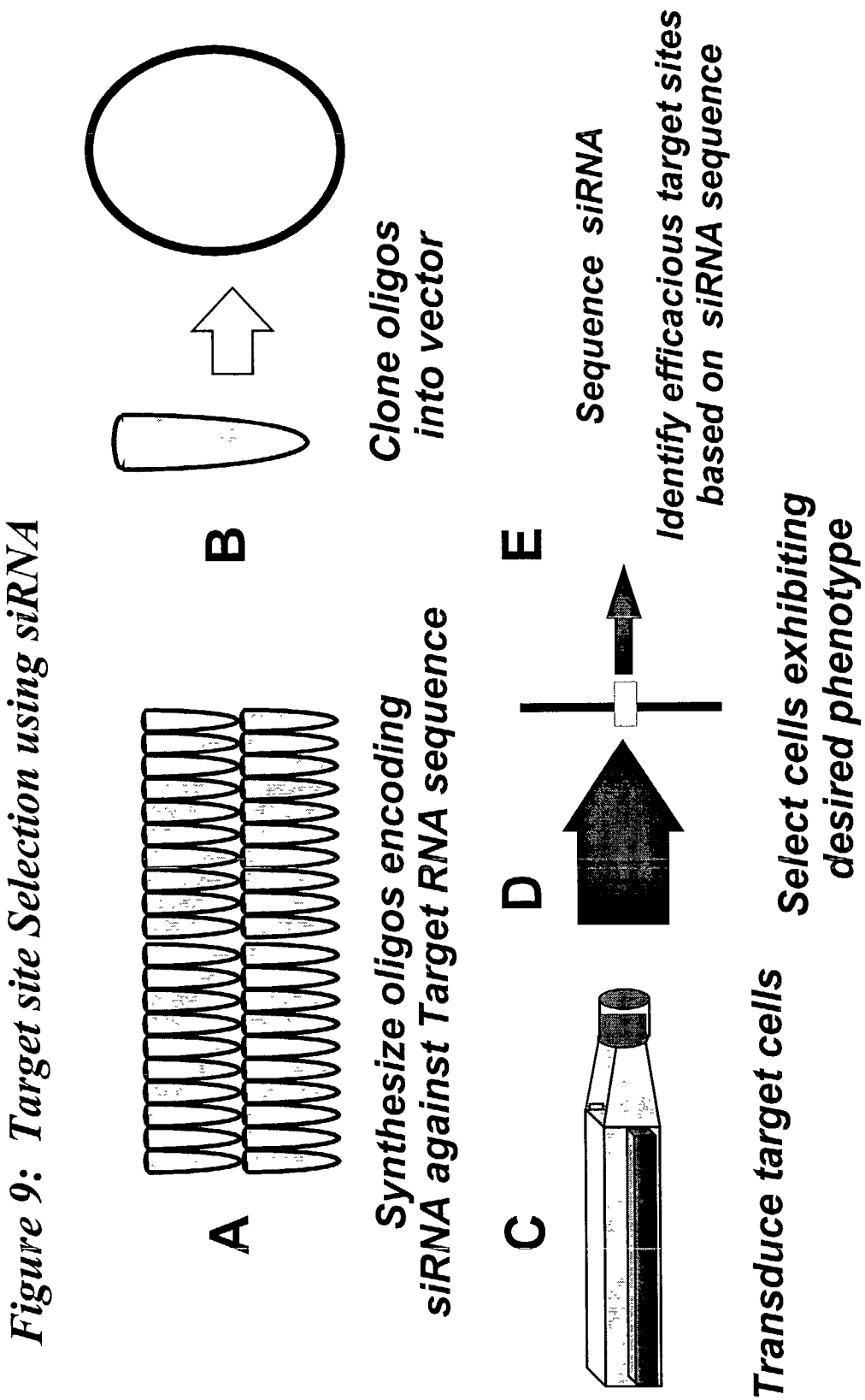


Figure 9: Target site Selection using siRNA

RNA INTERFERENCE MEDIATED INHIBITION OF HIV GENE EXPRESSION USING SHORT INTERFERING RNA

PRIORITY

[0001] This application claims the benefit of U.S. Application serial No. 60/294,140, filed May 29, 2001 and U.S. Application No. 60/398,036 filed Jul. 23, 2002. This application claims priority to U.S. Application Ser. No. 10/157,580 filed May 29, 2002.

BACKGROUND OF THE INVENTION

[0002] The present invention concerns methods and reagents useful in modulating HIV gene expression in a variety of applications, including use in therapeutic, diagnostic, target validation, and genomic discovery applications. Specifically, the invention relates to short interfering nucleic acid molecules capable of mediating RNA interference (RNAi) against HIV expression.

[0003] The following is a discussion of relevant art pertaining to RNAi. The discussion is provided only for understanding of the invention that follows. The summary is not an admission that any of the work described below is prior art to the claimed invention.

[0004] RNA interference refers to the process of sequence-specific post transcriptional gene silencing in animals mediated by short interfering RNAs (siRNA) (Fire et al., 1998, *Nature,* 391, 806). The corresponding process in plants is commonly referred to as post transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post transcriptional gene silencing is thought to be an evolutionarily conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire et al., 1999, *Trends Genet.,* 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double stranded RNAs (dsRNA) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

[0005] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNA) (Berstein et al., 2001, *Nature,* 409, 363). Short interfering RNAs derived from dicer activity are typically about 21-23 nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21 and 22 nucleotide small temporal RNAs (stRNA) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, *Science,* 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single stranded RNA having sequence complimentary to the antisense strand of the siRNA duplex. Cleavage of the target RNA takes place in the middle of the region complementary to the antisense strand of the siRNA duplex (Elbashir et al., 2001, *Genes Dev.,* 15, 188).

[0006] Short interfering RNA mediated RNAi has been studied in a variety of systems. Fire et al., 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. elegans.* Wianny and Goetz, 1999, *Nature Cell Biol.,* 2, 70, describe RNAi mediated by dsRNA in mouse embryos. Hammond et al., 2000, *Nature,* 404, 293, describe RNAi in Drosophila cells transfected with dsRNA. Elbashir et al., 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates (Elbashir et al., 2001, *EMBO J.,* 20, 6877) has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two nucleotide 3'-overhangs. Furthermore, complete substitution of one or both siRNA strands with 2'-deoxy (2'-H) or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of the 3'-terminal siRNA overhang nucleotides with deoxy nucleotides (2'-H) was shown to be tolerated. Single mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir et al., 2001, *EMBO J.,* 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen et al., 2001, *Cell,* 107, 309).

[0007] Studies have shown that replacing the 3'-overhanging segments of a 21-mer siRNA duplex having 2 nucleotide 3' overhangs with deoxyribonucleotides does not have an adverse effect on RNAi activity. Replacing up to 4 nucleotides on each end of the siRNA with deoxyribonucleotides has been reported to be well tolerated whereas complete substitution with deoxyribonucleotides results in no RNAi activity (Elbashir et al., 2001, *EMBO J.,* 20, 6877). In addition, Elbashir et al., supra, also report that substitution of siRNA with 2'-O-methyl nucleotides completely abolishes RNAi activity. Li et al., International PCT Publication No. WO 00/44914, and Beach et al., International PCT Publication No. WO 01/68836 both suggest that siRNA "may include modifications to either the phosphate-sugar back bone or the nucleoside to include at least one of a nitrogen or sulfur heteroatom", however neither application teaches to what extent these modifications are tolerated in siRNA molecules nor provide any examples of such modified siRNA. Kreutzer and Limmer, Canadian Patent Application No. 2,359,180, also describe certain chemical modifications for use in dsRNA constructs in order to counteract activation of double stranded-RNA-dependent protein kinase PKR, specifically 2'-amino or 2'-O-methyl nucleotides, and nucleotides containing a 2'-O or 4'-C methylene bridge. However, Kreutzer and Limmer similarly fail to show to what extent these modifications are tolerated in siRNA molecules nor do they provide any examples of such modified siRNA.

[0008] Parrish et al., 2000, *Molecular Cell,* 6, 1977-1087, tested certain chemical modifications targeting the unc-22 gene in *C. elegans* using long (>25 nt) siRNA transcripts. The authors describe the introduction of thiophosphate residues into these siRNA transcripts by incorporating thiophosphate nucleotide analogs with T7 and T3 RNA polymerase and observed that "RNAs with two [phosphorothioate] modified bases also had substantial decreases in effectiveness as RNAi triggers (data not shown); [phosphorothioate] modification of more than two residues greatly destabilized the RNAs in vitro and we were not able to assay interference activities." Id. at 1081. The authors also tested certain modifications at the 2'-position of the nucleotide sugar in the long siRNA transcripts and observed that substituting deoxynucleotides for ribonucleotides "produced a substantial decrease in interference activity", especially in the case of Uridine to Thymidine and/or Cytidine to deoxy-Cytidine substitutions. Id. In addition, the authors tested certain base modifications, including substituting 4-thiouracil, 5-bromouracil, 5-iodouracil, 3-(aminoallyl)uracil for uracil, and inosine for guanosine in sense and antisense strands of the siRNA, and found that whereas 4-thiouracil and 5-bromouracil were all well tolerated, inosine "produced a substantial decrease in interference activity" when incorporated in either strand. Incorporation of 5-iodouracil and 3-(aminoallyl)uracil in the antisense strand resulted in substantial decrease in RNAi activity as well.

[0009] Beach et al., International PCT Publication No. WO 01/68836, describes specific methods for attenuating gene expression using endogenously derived dsRNA. Tuschl et al., International PCT Publication No. WO 01/75164, describes a Drosophila in vitro RNAi system and the use of specific siRNA molecules for certain functional genomic and certain therapeutic applications; although Tuschl, 2001, *Chem. Biochem.,* 2, 239-245, doubts that RNAi can be used to cure genetic diseases or viral infection due "to the danger of activating interferon response". Li et al., International PCT Publication No. WO 00/44914, describes the use of specific dsRNAs for use in attenuating the expression of certain target genes. Zernicka-Goetz et al., International PCT Publication No. WO 01/36646, describes certain methods for inhibiting the expression of particular genes in mammalian cells using certain dsRNA molecules. Fire et al., International PCT Publication No. WO 99/32619, describes particular methods for introducing certain dsRNA molecules into cells for use in inhibiting gene expression. Plaetinck et al., International PCT Publication No. WO 00/01846, describes certain methods for identifying specific genes responsible for conferring a particular phenotype in a cell using specific dsRNA molecules. Mello et al., International PCT Publication No. WO 01/29058, describes the identification of specific genes involved in dsRNA mediated RNAi. Deschamps Depaillette et al., International PCT Publication No. WO 99/07409, describes specific compositions consisting of particular dsRNA molecules combined with certain anti-viral agents. Driscoll et al., International PCT Publication No. WO 01/49844, describes specific DNA constructs for use in facilitating gene silencing in targeted organisms. Parrish et al., 2000, *Molecular Cell,* 6, 1977-1087, describes specific chemically modified siRNA constructs targeting the unc-22 gene of *C. elegans.* Tuschl et al., International PCT Publication No. WO 02/44321, describe certain synthetic siRNA constructs.

[0010] Acquired immunodeficiency syndrome (AIDS) is thought to be caused by infection with the human immunodeficiency virus, for example HIV-1. Draper et al., U.S. Pat. Nos. 6,159,692, 5,972,704, 5,693,535, and International PCT Publication Nos. WO 93/23569 and WO 95/04818, describes enzymatic nucleic acid molecules targeting HIV. Novina et al., 2002, *Nature Medicine,* advance online publication, doi:10.1039/nm725, 1-6, describes certain siRNA constructs targeting HIV-1 infection. Lee et al., 2002, *Nature Biotechnology,* 19, 500-505, describes certain siRNA targeted against HIV-1 rev.

SUMMARY OF THE INVENTION

[0011] This invention relates to compounds, compositions, and methods useful for modulating human immunodeficiency virus (HIV) function and/or gene expression in a cell by RNA interference (RNAi) using short interfering RNA (siRNA). In particular, the instant invention features siRNA molecules and methods to modulate the expression of HIV RNA. The siRNA of the invention can be unmodified or chemically modified. The siRNA of the instant invention can be chemically synthesized, expressed from a vector or enzymatically synthesized. The instant invention also features various chemically modified synthetic short interfering RNA (siRNA) molecules capable of modulating HIV gene expression/activity in cells by RNA inference (RNAi). The use of chemically modified siRNA is expected to improve various properties of native siRNA molecules through increased resistance to nuclease degradation in vivo and/or improved cellular uptake. The siRNA molecules of the instant invention provide useful reagents and methods for a variety of therapeutic, diagnostic, agricultural, target validation, genomic discovery, genetic engineering and pharmacogenomic applications.

[0012] In one embodiment, the invention features one or more siRNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding HIV and/or HIV polypeptides. Specifically, the present invention features siRNA molecules that modulate the expression of HIV, for example HIV-1, HIV-2, and related viruses such as FIV-1 and SIV-1; or a HIV gene, for example LTR, nef, vif, tat, or rev. In particular embodiments, the invention features nucleic acid-based molecules and methods that modulate the expression of HIV-1 encoded genes, for example (Genbank Accession No. AJ302647); HIV-2 gene, for example (Genbank Accession No. NC_001722), FIV-1, for example (Genbank Accession No. NC_001482), SIV-1, for example (Genbank Accession No. M66437), LTR, for example included in (Genbank Accession No. AJ302647), nef, for example included in (Genbank Accession No. AJ302647), vif, for example included in (Genbank Accession No. AJ302647), tat, for example included in (Genbank Accession No. AJ302647), and rev, for example included in (Genbank Accession No. AJ302647).

[0013] In another embodiment, the invention features one or more siRNA molecules and methods that independently or in combination modulate the expression of gene(s) encoding the HIV-1 envelope glycoprotein (env, for example Genbank accession number NC_001802), such as to inhibit CD4 receptor mediated fusion of HIV-1. In particular, the present invention describes the selection and function of siRNA molecules capable of modulating HIV-1 envelope glycoprotein expression, for example expression of the

gp120 and gp41 subunits of HIV-1 envelope glycoprotein. These siRNA molecules can be used to treat diseases and disorders associated with HIV infection, or as a prophylactic measure to prevent HIV-1 infection.

[0014] In one embodiment, the invention features one or more siRNA molecules and methods that independently or in combination modulate the expression of genes representing cellular targets for HIV infection, such as cellular receptors, cell surface molecules, cellular enzymes, cellular transcription factors, and/or cytokines, second messengers, and cellular accessory molecules.

[0015] Non-limiting examples of such cellular receptors involved in HIV infection contemplated by the instant invention include CD4 receptors, CXCR4 (also known as Fusin; LESTR; NPY3R, such as Genbank Accession No. NM_003467),CCR5 (also known as CKR-5; CMKRB5 such as Genbank Accession No. NM_000579), CCR3 (also known as CC-CKR-3; CKR-3; CMKBR3, such as Genbank Accession No. NM_001837), CCR2 (also known as CCR2b; CMKBR2, such as Genbank Accession Nos. NM_000647 and NM_000648), CCR1 (also known as CKR1; CMKBR1, such as Genbank Accession No. NM_001295), CCR4 (also known as CKR-4, such as Genbank Accession No. NM_005508), CCR8 (also known as ChemR1; TER1; CMKBR8, such as Genbank Accession No. NM_005201), CCR9 (also known as D6, such as Genbank Accession Nos. NM_006641 and NM_031200), CXCR2 (also known as IL-8RB, such as Genbank Accession No. NM_001557), STRL33 (also known as Bonzo; TYMSTR, such as Genbank Accession No. NM_006564), US28, V28 (also known as CMKBRL1; CX3CR1; GPR13, such as Genbank Accession No. NM_001337), gpr1 (also known as GPR1, such as Genbank Accession No. NM_005279), gpr15 (also known as BOB; GPR15, such as Genbank Accession No. NM_005290), Apj (also known as angiotensin-receptor-like; AGTRL1, such as Genbank Accession No. NM_005161), and ChemR23 receptors (such as Genbank Accession No. NM_004072).

[0016] Non-limiting examples of cell surface molecules involved in HIV infection contemplated by the instant invention include Heparan Sulfate Proteoglycans, HSPG2 (such as Genbank Accession No. NM_005529), SDC2 (such as Genbank Accession Nos. AK025488, J04621, J04621), SDC4 (such as Genbank Accession No. NM_002999), GPC1 (such as Genbank Accession No. NM_002081), SDC3 (such as Genbank Accession No. NM_014654), SDC1 (such as Genbank Accession No. NM_002997), Galactoceramides, (such as Genbank Accession Nos. NM_000153, NM_003360, NM_001478.2, NM_004775, and NM_004861) and Erythrocyte-expressed Glycolipids (such as Genbank Accession Nos. NM_003778, NM_003779, NM_003780, NM_030587, and NM_001497).

[0017] Non-limiting examples of cellular enzymes involved in HIV infection contemplated by the invention include N-myristoyltransferase (NMT1, such as Genbank Accession No. NM_021079, and NMT2, such as Genbank Accession No. NM_004808), Glycosylation Enzymes (such as Genbank Accession Nos. NM_000303, NM_013339, NM_003358, NM_005787, NM_002408, NM_002676, NM_002435), NM_002409, NM_006122, NM_002372, NM_006699), NM_005907, NM_004479, NM_000150, NM_005216 and NM_005668), gp-160 Processing Enzymes (such as PCSK5, Genbank Accession No. NM_006200), Ribonucleotide Reductase (such as Genbank Accession Nos. NM_001034, NM_001033, AB036063, AB036063, AB036532, AK001965, AK001965, AK023605, AL137348, and AL137348), and Polyamine Biosynthesis enzymes (such as Genbank Accession Nos. NM_002539, NM_003132 and NM_001634).

[0018] Non-limiting examples of cellular transcription factors involved in HIV infection contemplated by the invention include SP-1 and NF-kappa B (such as NFKB2, Genbank Accession No. NM_002502, RELA, Genbank Accession No. NM_021975, and NFKB1 Genbank Accession No. NM_003998). Non-limiting examples of cytokines and second messengers involved in HIV infection contemplated by the invention include Tumor Necrosis Factor-a (TNF-a, such as Genbank Accession No. NM_000594), Interleukin 1a (IL-1a, such as Genbank Accession No. NM_000575), Interleukin 6 (IL-6, such as Genbank Accession No. NM_000600), Phospholipase C (such as Genbank Accession No. NM_000933) and Protein Kinase C (such as Genbank Accession No. NM_006255). Non-limiting examples of cellular accessory molecules involved in HIV infection contemplated by the invention include, Cyclophilins, (such as PPID, Genbank Accession No. NM_005038, PPIA, Genbank Accession No. NM_021130, PPIE, Genbank Accession No. NM_006112, PPIB, Genbank Accession No. NM_000942, PPIF Genbank Accession No. NM_005729, PPIG Genbank Accession No. NM_004792, and PPIC, Genbank Accession No. NM_000943), MAP-Kinase (Mitogen Activated Protein Kinase, such as MAPK1 Genbank Accession Nos. NM_002745 and NM_138957), and ERK-Kinase (Extracellular Signal-Regulated Kinase).

[0019] The description below of the various aspects and embodiments is provided with reference to the exemplary HIV-1 gene, referred to herein as HIV. However, the various aspects and embodiments are also directed to other genes which encode HIV polypeptides and/or similar viruses to HIV, as well as cellular targets as described herein. Those additional genes can be analyzed for target sites using the methods described for HIV. Thus, the inhibition and the effects of such inhibition of the other genes can be performed as described herein.

[0020] Due to the high sequence variability of the HIV genome, selection of nucleic acid molecules for broad therapeutic applications would likely involve the conserved regions of the HIV genome. Specifically, the present invention describes nucleic acid molecules that cleave the conserved regions of the HIV genome. Therefore, one nucleic acid molecule can be designed to cleave all the different isolates of HIV. Nucleic acid molecules designed against conserved regions of various HIV isolates can enable efficient inhibition of HIV replication in diverse subject populations and can ensure the effectiveness of the nucleic acid molecules against HIV quasi species which evolve due to mutations in the non-conserved regions of the HIV genome.

[0021] In one embodiment, the invention features a siRNA molecule that down regulates expression of a HIV gene by RNA interference, for example, wherein the HIV gene comprises HIV encoding sequence.

[0022] A siRNA molecule can be adapted for use to treat HIV infection or acquired immunodeficiency syndrome

(AIDS). A siRNA molecule can comprise a sense region and an antisense region and wherein said antisense region comprises sequence complementary to a HIV RNA sequence and the sense region comprises sequence complementary to the antisense region. A siRNA molecule can be assembled from two nucleic acid fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siRNA molecule. The sense region and antisense region can be covalently connected via a linker molecule. The linker molecule can be a polynucleotide linker or a non-nucleotide linker.

[0023] In one embodiment, the invention features a siRNA molecule having RNAi activity against HIV-1 RNA, wherein the siRNA molecule comprises a sequence complimentary to any RNA having HIV-1 encoding sequence, for example Genbank Accession No. AJ302647. In another embodiment, the invention features a siRNA molecule having RNAi activity against HIV-2 RNA, wherein the siRNA molecule comprises a sequence complimentary to any RNA having HIV-2 encoding sequence, for example Genbank Accession No. NC_001722. In another embodiment, the invention features a siRNA molecule having RNAi activity against FIV-1 RNA, wherein the siRNA molecule comprises a sequence complimentary to any RNA having FIV-1 encoding sequence, for example Genbank Accession No. NC_001482. In another embodiment, the invention features a siRNA molecule having RNAi activity against SIV-1 RNA, wherein the siRNA molecule comprises a sequence complimentary to any RNA having SIV-1 encoding sequence, for example Genbank Accession No. M66437.

[0024] In another embodiment, the invention features a siRNA molecule comprising sequences selected from the group consisting of SEQ ID NOs: 1-1476. A siRNA molecule can comprise and antisense region that comprises sequence complementary to sequence having any of SEQ ID NOs. 1-738. The antisense region can comprises sequence having any of SEQ ID NOs. 739-1476. The sense region can comprise sequence having any of SEQ ID NOs. 1-738. The sequences shown in SEQ ID NO:1-1476 are not limiting. A siRNA molecule of the invention can comprise any contiguous HIV sequences (e.g., about 19 contiguous HIV nucleotides).

[0025] In yet another embodiment, the invention features a siRNA molecule comprising a sequence complementary to a sequence comprising Genbank Accession Nos. AJ302647 (HIV-1), NC_001722 (HIV-2), NC_001482 (FIV-1) and/or M66437 (SIV-1).

[0026] In one embodiment, a siRNA molecule of the invention has RNAi activity that modulates expression of RNA encoded by a HIV gene.

[0027] A sense region of a siRNA molecule of the invention can comprise a 3'-terminal overhang and the antisense region can comprises a 3'-terminal overhang. The 3'-terminal overhangs each can comprise about 2 nucleotides. The antisense region 3'-terminal nucleotide overhang can be complementary to a HIV RNA.

[0028] In one embodiment, nucleic acid molecules of the invention that act as mediators of the RNA interference gene silencing response are double stranded RNA molecules. In another embodiment, the siRNA molecules of the invention consist of duplexes containing about 19 base pairs between oligonucleotides comprising about 19 to about 25 nucleotides, for example, about 19, 20, 21, 22, 23, 24 or 25 nucleotides. In yet another embodiment, siRNA molecules of the invention comprise duplexes with overhanging ends of 1-3 (i.e., 1, 2 or 3) nucleotides, for example 21 nucleotide duplexes with 19 base pairs and 2 nucleotide 3'-overhangs. These nucleotide overhangs in the antisense strand are optionally complimentary to the target sequence.

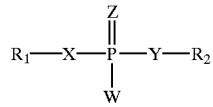
[0029] In one embodiment, the invention features one or more chemically modified siRNA constructs having specificity for HIV expressing nucleic acid molecules. Non-limiting examples of such chemical modifications include without limitation phosphorothioate internucleotide linkages, 2'-O-methyl ribonucleotides, 2'-O-methyl modified pyrimidine nucleotides, 2'-deoxy-2'-fluoro ribonucleotides, 2'-deoxy-2'-fluoro modified pyrimidine nucleotides, "universal base" nucleotides, 5-C-methyl nucleotides, and inverted deoxyabasic residue incorporation. These chemical modifications, when used in various siRNA constructs, are shown to preserve RNAi activity in cells while at the same time, dramatically increasing the serum stability of these compounds. Furthermore, contrary to the data published by Parrish et al., supra, applicant demonstrates that multiple (greater than one) phosphorothioate substitutions are well tolerated and confer substantial increases in serum stability for modified siRNA constructs. Chemical modifications of the siRNA constructs can also be used to improve the stability of the interaction with target RNA sequence and to improve nuclease resistance.

[0030] In one embodiment of the invention a siRNA molecule has an antisense region comprising a phosphorothioate internucleotide linkage at the 3' end of said antisense region. An antisense region can comprise between about one and about five phosphorothioate internucleotide linkages at the 5' end of said antisense region. The 3'-terminal nucleotide overhangs can comprise ribonucleotides or deoxyribonucleotides that are chemically modified at a nucleic acid sugar, base, or backbone. The 3'-terminal nucleotide overhangs can comprise one or more universal base ribonucleotides. The 3'-terminal nucleotide overhangs can comprise one or more acyclic nucleotides.

[0031] In another embodiment of the invention, an expression vector comprising a nucleic acid sequence encoding at least one siRNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. Another embodiment of the invention comprises a mammalian cell comprising an expression vector comprising a nucleic acid sequence encoding at least one siRNA molecule of the invention in a manner that allows expression of the nucleic acid molecule. The mammalian cell can be a human cell. The expression vector can comprise a siRNA molecule that comprises a sense region and an antisense region and wherein said antisense region comprises sequence complementary to a HIV RNA sequence and the sense region comprises sequence complementary to the antisense region. The expression vector can comprise a siRNA molecule that comprises two distinct strands having complementarity sense and antisense regions. The expression vector can comprise a siRNA molecule that comprises a single strand having complementary sense and antisense regions. In a non-limiting example, the introduction of chemically modified nucleotides into nucleic acid molecules will provide a powerful tool in overcoming potential limitations of in vivo

stability and bioavailability inherent to native RNA molecules that are delivered exogenously. For example, the use of chemically modified nucleic acid molecules can enable a lower dose of a particular nucleic acid molecule for a given therapeutic effect since chemically modified nucleic acid molecules tend to have a longer half-life in serum. Furthermore, certain chemical modifications can improve the bioavailability of nucleic acid molecules by targeting particular cells or tissues and/or improving cellular uptake of the nucleic acid molecule. Therefore, even if the activity of a chemically modified nucleic acid molecule is reduced as compared to a native nucleic acid molecule, for example when compared to an all RNA nucleic acid molecule, the overall activity of the modified nucleic acid molecule can be greater than the native molecule due to improved stability and/or delivery of the molecule. Unlike native unmodified siRNA, chemically modified siRNA can also minimize the possibility of activating interferon activity in humans.

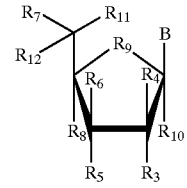
[0032] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises one or more nucleotides comprising a backbone modified internucleotide linkage having Formula I:



[0033] wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally occurring or chemically modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl, and wherein W, X, Y and Z are not all O.

[0034] The chemically modified internucleotide linkages having Formula I, for example wherein any Z, W, X, and/or Y independently comprises a sulphur atom, can be present in one or both oligonucleotide strands of the siRNA duplex, for example in the sense strand, antisense strand, or both strands. The siRNA molecules of the invention can comprise one or more chemically modified internucleotide linkages having Formula I at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand, antisense strand, or both strands. For example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, about 1, 2, 3, 4, 5 or more chemically modified internucleotide linkages having Formula I at the 5'-end of the sense strand, antisense strand, or both strands. In another non-limiting example, an exemplary siRNA molecule of the invention can comprise one or more pyrimidine nucleotides with chemically modified internucleotide linkages having Formula I in the sense strand, antisense strand, or both strands. In yet another non-limiting example, an exemplary siRNA molecule of the invention can comprise one or more purine nucleotides with chemically modified internucleotide linkages having Formula I in the sense strand, antisense strand, or both strands. In another embodiment, a siRNA molecule of the invention having internucleotide linkage(s) of Formula I also comprises a chemically modified nucleotide or non-nucleotide having any of Formulae II, III, V, or VI.

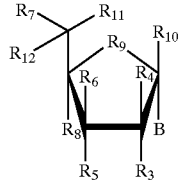
[0035] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises one or more nucleotides or non-nucleotides having Formula II:



[0036] wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, 0-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to form a stable duplex with RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be employed to form a stable duplex with RNA.

[0037] The chemically modified nucleotide or non-nucleotide of Formula II can be present in one or both oligonucleotide strands of the siRNA duplex, for example in the sense strand, antisense strand, or both strands. The siRNA molecules of the invention can comprise one or more chemically modified nucleotide or non-nucleotide of Formula II at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand, antisense strand, or both strands. For example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, about 1, 2, 3, 4, 5 or more chemically modified nucleotide or non-nucleotide of Formula II at the 5'-end of the sense strand, antisense strand, or both strands. In anther non-limiting example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, 1, 2, 3, 4, 5 or more chemically modified nucleotide or non-nucleotide of Formula II at the 3'-end of the sense strand, antisense strand, or both strands.

[0038] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises one or more nucleotides or non-nucleotides having Formula III:

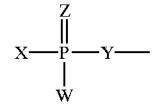


[0039] wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S═O, CHF, or CF2, and B is a nucleosidic base such as adenine, guanine, uracil, cytosine, thymine, 2-aminoadenosine, 5-methylcytosine, 2,6-diaminopurine, or any other non-naturally occurring base that can be employed to form a stable duplex with RNA or a non-nucleosidic base such as phenyl, naphthyl, 3-nitropyrrole, 5-nitroindole, nebularine, pyridone, pyridinone, or any other non-naturally occurring universal base that can be employed to form a stable duplex with RNA.

[0040] The chemically modified nucleotide or non-nucleotide of Formula III can be present in one or both oligonucleotide strands of the siRNA duplex, for example in the sense strand, antisense strand, or both strands. The siRNA molecules of the invention can comprise one or more chemically modified nucleotide or non-nucleotide of Formula III at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand, antisense strand, or both strands. For example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, about 1, 2, 3, 4, 5 or more chemically modified nucleotide or non-nucleotide of Formula III at the 5'-end of the sense strand, antisense strand, or both strands. In anther non-limiting example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, about 1, 2, 3, 4, 5 or more chemically modified nucleotide or non-nucleotide of Formula III at the 3'-end of the sense strand, antisense strand, or both strands.

[0041] In another embodiment, a siRNA molecule of the invention comprises a nucleotide having Formula II or III, wherein the nucleotide having Formula II or III is in an inverted configuration. For example, the nucleotide having Formula II or III is connected to the siRNA construct in a 3',3', 3'–2', 2'–3', or 5',5' configuration, such as at the 3'-end, 5'-end, or both 3' and 5' ends of one or both siRNA strands.

[0042] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises a 5'-terminal phosphate group having Formula IV:



[0043] wherein each X and Y is independently O, S, N, alkyl, substituted alkyl, or alkylhalo; each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, aralkyl, or alkylhalo; and wherein W, X, Y and Z are not all O.

[0044] In one embodiment, the invention features a siRNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complimentary strand, for example a strand complimentary to HIV RNA, wherein the siRNA molecule comprises an all RNA siRNA molecule. In another embodiment, the invention features a siRNA molecule having a 5'-terminal phosphate group having Formula IV on the target-complimentary strand wherein the siRNA molecule also comprises 1-3 (i.e., 1, 2 or 3) nucleotide 3'-overhangs having between about 1 and about 4, for example, about 1, 2, 3 or 4 deoxyribonucleotides on the 3'-end of one or both strands. In another embodiment, a 5'-terminal phosphate group having Formula IV is present on the target-complimentary strand of a siRNA molecule of the invention, for example a siRNA molecule having chemical modifications having Formula I, Formula II and/or Formula III.

[0045] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises one or more phosphorothioate internucleotide linkages. For example, in a non-limiting example, the invention features a chemically modified short interfering RNA (siRNA) having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in one siRNA strand. In yet another embodiment, the invention features a chemically modified short interfering RNA (siRNA) individually having about 1, 2, 3, 4, 5, 6, 7, 8 or more phosphorothioate internucleotide linkages in both siRNA strands. The phosphorothioate internucleotide linkages can be present in one or both oligonucleotide strands of the siRNA duplex, for example in the sense strand, antisense strand, or both strands. The siRNA molecules of the invention can comprise one or more phosphorothioate internucleotide linkages at the 3'-end, 5'-end, or both 3' and 5'-ends of the sense strand, antisense strand, or both strands. For example, an exemplary siRNA molecule of the invention can comprise between about 1 and about 5 or more, for example, about 1, 2, 3, 4, 5 or more phosphorothioate internucleotide linkages at the 5'-end of the sense strand, antisense strand, or both strands. In another non-limiting example, an exemplary siRNA molecule of the invention can comprise one or more pyrimidine phosphorothioate internucleotide linkages in the sense strand, antisense strand, or both strands. In yet another non-limiting example, an exemplary siRNA molecule of the invention can comprise one or more purine phosphorothioate internucleotide linkages in the sense strand, antisense strand, or both strands.

[0046] In one embodiment, the invention features a siRNA molecule, wherein the sense strand comprises one or more,

for example about 1, 2, 3, 4, 5, 6, 7, 8 , 9, or 10 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more, for example, about 1, 2, 3, 4, 5 or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the sense strand; and wherein the antisense strand comprises any of between 1 and 10, specifically about 1, 2, 3, 4, 5, 6, 7, 8 , 9, or 10 phosphorothioate internucleotide linkages, and/or one or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 pyrimidine nucleotides of the sense and/or antisense siRNA stand are chemically modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3', 5', or both 3' and 5'-ends, being present in the same or different strand.

[0047] In another embodiment, the invention features a siRNA molecule, wherein the sense strand comprises between 1 and 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more, for example, about 1, 2, 3, 4, 5 or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the sense strand; and wherein the antisense strand comprises any of between 1 and 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more, for example, about 1, 2, 3, 4, 5 or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 pyrimidine nucleotides of the sense and/or antisense siRNA stand are chemically modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without between 1 and 5, for example about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3', 5', or both 3' and 5'-ends, being present in the same or different strand.

[0048] In one embodiment, the invention features a siRNA molecule, wherein the antisense strand comprises one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more, for example, 1, 2, 3, 4, 5 or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the sense strand; and wherein the antisense strand comprises any of between 1 and 10, specifically about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 pyrimidine nucleotides of the sense and/or antisense siRNA stand are chemically modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3', 5', or both 3' and 5'-ends, being present in the same or different strand.

[0049] In another embodiment, the invention features a siRNA molecule, wherein the antisense strand comprises between 1 and 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the sense strand; and wherein the antisense strand comprises any of between 1 and 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages, and/or one or more, for example, about 1, 2, 3, 4, 5 or more 2'-deoxy, 2'-O-methyl, 2'-deoxy-2'-fluoro, and/or one or more, for example, about 1, 2, 3, 4, 5 or more universal base modified nucleotides, and optionally a terminal cap molecule at the 3', 5', or both 3' and 5'-ends of the antisense strand. In another embodiment, one or more, for example about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 pyrimidine nucleotides of the sense and/or antisense siRNA stand are chemically modified with 2'-deoxy, 2'-O-methyl and/or 2'-deoxy-2'-fluoro nucleotides, with or without between 1 and 5, for example about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages and/or a terminal cap molecule at the 3', 5', or both 3' and 5'-ends, being present in the same or different strand.

[0050] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule having between about 1 and 5, specifically about 1, 2, 3, 4, or 5 phosphorothioate internucleotide linkages in each strand of the siRNA molecule.

[0051] In another embodiment, the invention features a siRNA molecule comprising 2'-5' internucleotide linkages. The 2'-5' internucleotide linkage(s) can be at the 5'-end, 3'-end, or both 5' and 3' ends of one or both siRNA sequence strands. In addition, the 2'-5' internucleotide linkage(s) can be present at various other positions within one or both siRNA sequence strands, for example, every internucleotide linkage of a pyrimidine nucleotide in one or both strands of the siRNA molecule can comprise a 2'-5' internucleotide linkage, or every internucleotide linkage of a purine nucleotide in one or both strands of the siRNA molecule can comprise a 2'-5' internucleotide linkage.

[0052] In another embodiment, a chemically modified siRNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically modified, wherein each strand is between about 18 and about 27, for example, about 18, 19, 20, 21, 22, 23, 24, 25, 26 or 27, nucleotides in length, wherein the duplex has between about 18 and about 23, for example, about 18, 19, 20, 21, 22, 23, base pairs, and wherein the chemical modification comprises a structure having Formula I, Formula II, Formula III and/or Formula IV. For example, an exemplary chemically

modified siRNA molecule of the invention comprises a duplex having two strands, one or both of which can be chemically modified with a chemical modification having Formula I, Formula II, Formula III, and/or Formula IV, wherein each strand consists of 21 nucleotides, each having 2 nucleotide 3'-overhangs, and wherein the duplex has 19 base pairs.

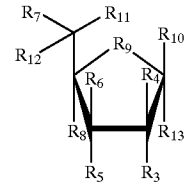
[0053] In another embodiment, a siRNA molecule of the invention comprises a single stranded hairpin structure, wherein the siRNA is between about 36 and about 70, for example, about 36, 40, 45, 50, 55, 60, 65, or 70, nucleotides in length having between about 18 and about 23, for example, about 18, 19, 20, 21, 22, or 23 base pairs, and wherein the siRNA can include a chemical modification comprising a structure having Formula I, Formula II, Formula III and/or Formula IV. For example, an exemplary chemically modified siRNA molecule of the invention comprises a linear oligonucleotide having between about 42 and about 50, for example, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides that is chemically modified with a chemical modification having Formula I, Formula II, Formula III, and/or Formula IV, wherein the linear oligonucleotide forms a hairpin structure having 19 base pairs and a 2 nucleotide 3'-overhang.

[0054] In another embodiment, a linear hairpin siRNA molecule of the invention contains a stem loop motif, wherein the loop portion of the siRNA molecule is biodegradable. For example, a linear hairpin siRNA molecule of the invention is designed such that degradation of the loop portion of the siRNA molecule in vivo can generate a double stranded siRNA molecule with 3'-overhangs, such as 3'-overhangs comprising about 2 nucleotides.

[0055] In another embodiment, a siRNA molecule of the invention comprises a circular nucleic acid molecule, wherein the siRNA is between about 38 and about 70, for example, about 38, 40, 45, 50, 55, 60, 65 or 70 nucleotides in length having between about 18 and about 23, for example, about 18, 19, 20, 21, 22 or 23 base pairs, and wherein the siRNA can include a chemical modification, which comprises a structure having Formula I, Formula II, Formula III and/or Formula IV. For example, an exemplary chemically modified siRNA molecule of the invention comprises a circular oligonucleotide having between about 42 and about 50, for example, 42, 43, 44, 45, 46, 47, 48, 49 or 50 nucleotides that is chemically modified with a chemical modification having Formula I, Formula II, Formula III, and/or Formula IV, wherein the circular oligonucleotide forms a dumbbell shaped structure having 19 base pairs and 2 loops.

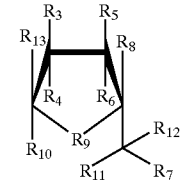
[0056] In another embodiment, a circular siRNA molecule of the invention contains two loop motifs, wherein one or both loop portions of the siRNA molecule is biodegradable. For example, a circular siRNA molecule of the invention is designed such that degradation of the loop portions of the siRNA molecule in vivo can generate a double stranded siRNA molecule with 3'-overhangs, such as 3'-overhangs comprising about 2 nucleotides.

[0057] In one embodiment, a siRNA molecule of the invention comprises one or more abasic residues, for example a compound having Formula V:



[0058] wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, 0-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2.

[0059] In one embodiment, a siRNA molecule of the invention comprises one or more inverted abasic residues, for example a compound having Formula VI:



[0060] wherein each R3, R4, R5, R6, R7, R8, R10, R11, R12, and R13 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S=O, CHF, or CF2, and either R2, R3, R8 or R13 serve as points of attachment to the siRNA molecule of the invention.

[0061] In another embodiment, a siRNA molecule of the invention comprises an abasic residue having Formula II or III, wherein the abasic residue having Formula II or III is connected to the siRNA construct in a 3',3', 3'–2', 2'–3', or 5', 5' configuration, such as that the 3'-end, 5'-end, or both 3' and 5' ends of one or both siRNA strands.

[0062] In one embodiment, a siRNA molecule of the invention comprises one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more locked nucleic acid (LNA) nucleotides, for example at the 5'-end, 3'-end, 5' and 3'-end, or any combination thereof, of the siRNA molecule.

[0063] In one embodiment, the invention features a chemically modified short interfering RNA (siRNA) molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein the chemical modification comprises a conjugate covalently attached to the siRNA molecule. In another embodiment, the conjugate is covalently attached to the siRNA molecule via a biodegradable linker. In one embodiment, the conjugate molecule is attached at the 3'-end of either the sense strand, antisense strand, or both strands of the siRNA. In another embodiment, the conjugate molecule is attached at the 5'-end of either the sense strand, antisense strand, or both strands of the siRNA. In yet another embodiment, the conjugate molecule is attached both the 3'-end and 5'-end of either the sense strand, antisense strand, or both strands of the siRNA, or any combination thereof. In one embodiment, a conjugate molecule of the invention comprises a molecule that facilitates delivery of a siRNA molecule into a biological system such as a cell. In another embodiment, the conjugate molecule attached to the siRNA is a poly ethylene glycol, human serum albumin, or a ligand for a cellular receptor that can mediate cellular uptake. Examples of specific conjugate molecules contemplated by the instant invention that can be attached to siRNA molecules are described in Vargeese et al., U.S. Serial No. 60/311,865, incorporated by reference herein.

[0064] In one embodiment, the invention features a siRNA molecule capable of mediating RNA interference (RNAi) against HIV inside a cell, wherein one or both strands of the siRNA comprise ribonucleotides at positions withing the siRNA that are critical for siRNA mediated RNAi in a cell. All other positions within the siRNA can include chemically modified nucleotides and/or non-nucleotides such as nucleotides and or non-nucleotides having Formula I, II, III, IV, V, or VI, or any combination thereof to the extent that the ability of the siRNA molecule to support RNAi activity in a cell is maintained.

[0065] In one embodiment, the invention features a method for modulating the expression of a HIV gene within a cell, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene; and (b) introducing the siRNA molecule into a cell under conditions suitable to modulate the expression of the HIV gene in the cell.

[0066] In one embodiment, the invention features a method for modulating the expression of a HIV gene within a cell, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene and wherein the sense strand sequence of the siRNA is identical to the complimentary sequence of the HIV RNA; and (b) introducing the siRNA molecule into a cell under conditions suitable to modulate the expression of the HIV gene in the cell.

[0067] In another embodiment, the invention features a method for modulating the expression of more than one HIV gene within a cell, comprising: (a) synthesizing siRNA molecules of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV genes; and (b) introducing the siRNA molecules into a cell under conditions suitable to modulate the expression of the HIV genes in the cell.

[0068] In another embodiment, the invention features a method for modulating the expression of more than one HIV gene within a cell, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene and wherein the sense strand sequence of the siRNA is identical to the complimentary sequence of the HIV RNA; and (b) introducing the siRNA molecules into a cell under conditions suitable to modulate the expression of the HIV genes in the cell.

[0069] In one embodiment, the invention features a method of modulating the expression of a HIV gene in a tissue explant, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene; (b) introducing the siRNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HIV gene in the tissue explant, and (c) optionally introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HIV gene in that organism.

[0070] In one embodiment, the invention features a method of modulating the expression of a HIV gene in a tissue explant, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene and wherein the sense strand sequence of the siRNA is identical to the complimentary sequence of the HIV RNA; (b) introducing the siRNA molecule into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HIV gene in the tissue explant, and (c) optionally introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HIV gene in that organism.

[0071] In another embodiment, the invention features a method of modulating the expression of more than one HIV gene in a tissue explant, comprising: (a) synthesizing siRNA molecules of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV genes; (b) introducing the siRNA molecules into a cell of the tissue explant derived from a particular organism under conditions suitable to modulate the expression of the HIV genes in the tissue explant, and (c) optionally introducing the tissue explant back into the organism the tissue was derived from or into another organism under conditions suitable to modulate the expression of the HIV genes in that organism.

[0072] In one embodiment, the invention features a method of modulating the expression of a HIV gene in an organism, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV gene; and (b) introducing the siRNA molecule into the organism under conditions suitable to modulate the expression of the HIV gene in the organism.

[0073] In another embodiment, the invention features a method of modulating the expression of more than one HIV

gene in an organism, comprising: (a) synthesizing siRNA molecules of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of the HIV genes; and (b) introducing the siRNA molecules into the organism under conditions suitable to modulate the expression of the HIV genes in the organism.

[0074] The siRNA molecules of the invention can be designed to inhibit HIV gene expression through RNAi targeting of a variety of RNA molecules. In one embodiment, the siRNA molecules of the invention are used to target various RNAs corresponding to a target gene. Non-limiting examples of such RNAs include messenger RNA (mRNA), alternate RNA splice variants of target gene(s), post-transcriptionally modified RNA of target gene(s), pre-mRNA of target gene(s), and/or RNA templates used for HIV activity. If alternate splicing produces a family of transcipts that are distinguished by usage of appropriate exons, the instant invention can be used to inhibit gene expression through the appropriate exons to specifically inhibit or to distinguish among the functions of gene family members. For example, a protein that contains an alternatively spliced transmembrane domain can be expressed in both membrane bound and secreted forms. Use of the invention to target the exon containing the transmembrane domain can be used to determine the functional consequences of pharmaceutical targeting of membrane bound as opposed to the secreted form of the protein. Non-limiting examples of applications of the invention relating to targeting these RNA molecules include therapeutic pharmaceutical applications, pharmaceutical discovery applications, molecular diagnostic and gene function applications, and gene mapping, for example using single nucleotide polymorphism mapping with siRNA molecules of the invention. Such applications can be implemented using known gene sequences or from partial sequences available from an expressed sequence tag (EST).

[0075] In another embodiment, the siRNA molecules of the invention are used to target conserved sequences corresponding to a gene family or gene families such as HIV genes. As such, siRNA molecules targeting multiple HIV targets can provide increased therapeutic effect. In addition, siRNA can be used to characterize pathways of gene function in a variety of applications. For example, the present invention can be used to inhibit the activity of target gene(s) in a pathway to determine the function of uncharacterized gene(s) in gene function analysis, mRNA function analysis, or translational analysis. The invention can be used to determine potential target gene pathways involved in various diseases and conditions toward pharmaceutical development. The invention can be used to understand pathways of gene expression involved in development, such as prenatal development, postnatal development and/or aging.

[0076] In one embodiment, siRNA molecule(s) and/or methods of the invention are used to inhibit the expression of gene(s) that encode RNA referred to by Genbank Accession number, for example HIV genes such as Genbank Accession Nos. AJ302647 (HIV-1), NC_001722 (HIV-2), NC_001482 (FIV-1) and/or M66437 (SIV-1). Such sequences are readily obtained using these Genbank Accession numbers.

[0077] In one embodiment, the invention features a method comprising: (a) analyzing the sequence of a RNA target encoded by a HIV gene; (b) synthesizing one or more sets of siRNA molecules having sequence complimentary to one or more regions of the RNA of (a); and (c) assaying the siRNA molecules of (b) under conditions suitable to determine RNAi targets within the target RNA sequence. In another embodiment, the siRNA molecules of (b) have strands of a fixed length, for example 23 nucleotides in length. In yet another embodiment, the siRNA molecules of (b) are of differing length, for example having strands of about 19 to about 25, for example, about 19, 20, 21, 22, 23, 24 or 25 nucleotides in length.

[0078] In one embodiment, the invention features a composition comprising a siRNA molecule of the invention, which can be chemically modified, in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a pharmaceutical composition comprising siRNA molecules of the invention, which can be chemically modified, targeting one or more genes in a pharmaceutically acceptable carrier or diluent. In another embodiment, the invention features a method for treating or preventing a disease or condition in a subject, comprising administering to the subject a composition of the invention under conditions suitable for the treatment or prevention of the disease or condition in the subject, alone or in conjunction with one or more other therapeutic compounds. In yet another embodiment, the invention features a method for reducing or preventing tissue rejection in a subject comprising administering to the subject a composition of the invention under conditions suitable for the reduction or prevention of tissue rejection in the subject.

[0079] In another embodiment, the invention features a method for validating a HIV gene target, comprising: (a) synthesizing a siRNA molecule of the invention, which can be chemically modified, wherein one of the siRNA strands includes a sequence complimentary to RNA of a HIV target gene; (b) introducing the siRNA molecule into a cell, tissue, or organism under conditions suitable for modulating expression of the HIV target gene in the cell, tissue, or organism; and (c) determining the function of the gene by assaying for any phenotypic change in the cell, tissue, or organism.

[0080] In one embodiment, the invention features a kit containing a siRNA molecule of the invention, which can be chemically modified, that can be used to modulate the expression of a HIV target gene in a cell, tissue, or organism. In another embodiment, the invention features a kit containing more than one siRNA molecule of the invention, which can be chemically modified, that can be used to modulate the expression of more than one HIV target gene in a cell, tissue, or organism.

[0081] In one embodiment, the invention features a cell containing one or more siRNA molecules of the invention, which can be chemically modified. In another embodiment, the cell containing a siRNA molecule of the invention is a mammalian cell. In yet another embodiment, the cell containing a siRNA molecule of the invention is a human cell.

[0082] In one embodiment, the synthesis of a siRNA molecule of the invention, which can be chemically modified, comprises: (a) synthesis of two complimentary strands of the siRNA molecule; (b) annealing the two complimentary strands together under conditions suitable to obtain a double stranded siRNA molecule. In another embodiment,

synthesis of the two complimentary strands of the siRNA molecule is by solid phase oligonucleotide synthesis. In yet another embodiment, synthesis of the two complimentary strands of the siRNA molecule is by solid phase tandem oligonucleotide synthesis.

[0083] In one embodiment, the invention features a method for synthesizing a siRNA duplex molecule comprising: (a) synthesizing a first oligonucleotide sequence strand of the siRNA molecule, wherein the first oligonucleotide sequence strand comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of the second oligonucleotide sequence strand of the siRNA; (b) synthesizing the second oligonucleotide sequence strand of siRNA on the scaffold of the first oligonucleotide sequence strand, wherein the second oligonucleotide sequence strand further comprises a chemical moiety than can be used to purify the siRNA duplex; (c) cleaving the linker molecule of (a) under conditions suitable for the two siRNA oligonucleotide strands to hybridize and form a stable duplex; and (d) purifying the siRNA duplex utilizing the chemical moiety of the second oligonucleotide sequence strand. In another embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions using an alkylamine base such as methylamine. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place concomitantly. In another embodiment, the chemical moiety of (b) that can used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group, which can be employed in a trityl-on synthesis strategy as described herein. In yet another embodiment, the chemical moiety, such as a dimethoxytrityl group, is removed during purification, for example using acidic conditions.

[0084] In a further embodiment, the method for siRNA synthesis is a solution phase synthesis or hybrid phase synthesis wherein both strands of the siRNA duplex are synthesized in tandem using a cleavable linker attached to the first sequence which acts a scaffold for synthesis of the second sequence. Cleavage of the linker under conditions suitable for hybridization of the separate siRNA sequence strands results in formation of the double stranded siRNA molecule.

[0085] In another embodiment, the invention features a method for synthesizing a siRNA duplex molecule comprising: (a) synthesizing one oligonucleotide sequence strand of the siRNA molecule, wherein the sequence comprises a cleavable linker molecule that can be used as a scaffold for the synthesis of another oligonucleotide sequence; (b) synthesizing a second oligonucleotide sequence having complementarity to the first sequence strand on the scaffold of (a), wherein the second sequence comprises the other strand of the double stranded siRNA molecule and wherein the second sequence further comprises a chemical moiety than can be used to isolate the attached oligonucleotide sequence; (c) purifying the product of (b) utilizing the chemical moiety of the second oligonucleotide sequence strand under conditions suitable for isolating the full length sequence comprising both siRNA oligonucleotide strands connected by the cleavable linker; and (d) under conditions suitable for the two siRNA oligonucleotide strands to hybridize and form a stable duplex. In another embodiment, cleavage of the linker molecule in (c) above takes place during deprotection of the oligonucleotide, for example under hydrolysis conditions. In another embodiment, cleavage of the linker molecule in (c) above takes place after deprotection of the oligonucleotide. In another embodiment, the method of synthesis comprises solid phase synthesis on a solid support such as controlled pore glass (CPG) or polystyrene, wherein the first sequence of (a) is synthesized on a cleavable linker, such as a succinyl linker, using the solid support as a scaffold. The cleavable linker in (a) used as a scaffold for synthesizing the second strand can comprise similar reactivity or differing reactivity as the solid support derivatized linker, such that cleavage of the solid support derivatized linker and the cleavable linker of (a) takes place either concomitantly or sequentially. In another embodiment, the chemical moiety of (b) that can used to isolate the attached oligonucleotide sequence comprises a trityl group, for example a dimethoxytrityl group.

[0086] In another embodiment, the invention features a method for making a double stranded siRNA molecule in a single synthetic process, comprising: (a) synthesizing an oligonucleotide having a first and a second sequence, wherein the first sequence is complimentary to the second sequence, and the first oligonucleotide sequence is linked to the second sequence via a cleavable linker, and wherein a terminal 5'-protecting group, for example a 5'-O-dimethoxytrityl group (5'-O-DMT) remains on the oligonucleotide having the second sequence; (b) deprotecting the oligonucleotide whereby the deprotection results in the cleavage of the linker joining the two oligonucleotide sequences; and (c) purifying the product of (b) under conditions suitable for isolating the double stranded siRNA molecule, for example using a trityl-on synthesis strategy as described herein.

[0087] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications, for example one or more chemical modifications having Formula I, II, III, IV, or V, that increases the nuclease resistance of the siRNA construct.

[0088] In another embodiment, the invention features a method for generating siRNA molecules with increased nuclease resistance comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having increased nuclease resistance.

[0089] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the sense and antisense strands of the siRNA construct.

[0090] In another embodiment, the invention features a method for generating siRNA molecules with increased binding affinity between the sense and antisense strands of the siRNA molecule comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b)

assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having increased binding affinity between the sense and antisense strands of the siRNA molecule.

[0091] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications described herein that modulates the binding affinity between the antisense strand of the siRNA construct and a complimentary target RNA sequence within a cell.

[0092] In another embodiment, the invention features a method for generating siRNA molecules with increased binding affinity between the antisense strand of the siRNA molecule and a complimentary target RNA sequence, comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having increased binding affinity between the antisense strand of the siRNA molecule and a complimentary target RNA sequence.

[0093] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications described herein that modulate the polymerase activity of a cellular polymerase capable of generating additional endogenous siRNA molecules having sequence homology to the chemically modified siRNA construct.

[0094] In another embodiment, the invention features a method for generating siRNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siRNA molecules having sequence homology to the chemically modified siRNA molecule comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules capable of mediating increased polymerase activity of a cellular polymerase capable of generating additional endogenous siRNA molecules having sequence homology to the chemically modified siRNA molecule.

[0095] In one embodiment, the invention features chemically modified siRNA constructs that mediate RNAi against HIV in a cell, wherein the chemical modifications do not significantly effect the interaction of siRNA with a target RNA molecule and/or proteins or other factors that are essential for RNAi in a manner that would decrease the efficacy of RNAi mediated by such siRNA constructs.

[0096] In another embodiment, the invention features a method for generating siRNA molecules with improved RNAi activity against HIV, comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved RNAi activity.

[0097] In yet another embodiment, the invention features a method for generating siRNA molecules with improved RNAi activity against a HIV target RNA, comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved RNAi activity against the target RNA.

[0098] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications described herein that modulates the cellular uptake of the siRNA construct.

[0099] In another embodiment, the invention features a method for generating siRNA molecules against HIV with improved cellular uptake, comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved cellular uptake.

[0100] In one embodiment, the invention features siRNA constructs that mediate RNAi against HIV, wherein the siRNA construct comprises one or more chemical modifications described herein that increases the bioavailability of the siRNA construct, for example by attaching polymeric conjugates such as polyethyleneglycol or equivalent conjugates that improve the pharmacokinetics of the siRNA construct, or by attaching conjugates that target specific tissue types or cell types in vivo. Non-limiting examples of such conjugates are described in Vargeese et al., U.S. Serial No. 60/311,865 incorporated by reference herein.

[0101] In one embodiment, the invention features a method for generating siRNA molecules of the invention with improved bioavailability, comprising (a) introducing a conjugate into the structure of a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved bioavailability. Such conjugates can include ligands for cellular receptors such as peptides derived from naturally occurring protein ligands, protein localization sequences including cellular ZIP code sequences, antibodies, nucleic acid aptamers, vitamins and other co-factors such as folate and N-acetylgalactosamine, polymers such as polyethyleneglycol (PEG), phospholipids, polyamines such as spermine or spermidine, and others.

[0102] In another embodiment, the invention features a method for generating siRNA molecules of the invention with improved bioavailability, comprising (a) introducing an excipient formulation to a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved bioavailability. Such excipients include polymers such as cyclodextrins, lipids, cationic lipids, polyamines, phospholipids, and others.

[0103] In another embodiment, the invention features a method for generating siRNA molecules of the invention with improved bioavailability, comprising (a) introducing nucleotides having any of Formula I-VI into a siRNA molecule, and (b) assaying the siRNA molecule of step (a) under conditions suitable for isolating siRNA molecules having improved bioavailability.

[0104] In another embodiment, polyethylene glycol (PEG) can be covalently attached to siRNA compounds of the present invention. The attached PEG can be any molecular weight, preferably from about 2,000 to about 50,000 daltons (Da).

[0105] The present invention can be used alone or as a component of a kit having at least one of the reagents necessary to carry out the in vitro or in vivo introduction of

RNA to test samples and/or subjects. For example, preferred components of the kit include the siRNA and a vehicle that promotes introduction of the siRNA. Such a kit can also include instructions to allow a user of the kit to practice the invention.

[0106] The term "short interfering RNA" or "siRNA" as used herein refers to any nucleic acid molecule capable of mediating RNA interference "RNAi" or gene silencing; see for example Bass, 2001, *Nature,* 411, 428-429; Elbashir et al., 2001, *Nature,* 411, 494-498; and Kreutzer et al., International PCT Publication No. WO 00/44895; Zernicka-Goetz et al., International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck et al., International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; and Li et al., International PCT Publication No. WO 00/44914. Non limiting examples of siRNA molecules of the invention are shown in **FIG. 6**. For example the siRNA can be a double stranded polynucleotide molecule comprising self complementary sense and antisense regions, wherein the antisense region comprises complementarity to a target nucleic acid molecule. The siRNA can be a single stranded hairpin polynucleotide having self complementary sense and antisense regions, wherein the antisense region comprises complementarity to a target nucleic acid molecule. The siRNA can be a circular single stranded polynucleotide having two or more loop structures and a stem comprising self complementary sense and antisense regions, wherein the antisense region comprises complementarity to a target nucleic acid molecule, and wherein the circular polynucleotide can be processed either in vivo or in vitro to generate an active siRNA capable of mediating RNAi. As used herein, siRNA molecules need not be limited to those molecules containing only RNA, but further encompasses chemically modified nucleotides and non-nucleotides..

[0107] By "modulate" is meant that the expression of the gene, or level of RNA molecule or equivalent RNA molecules encoding one or more proteins or protein subunits, or activity of one or more proteins or protein subunits is up regulated or down regulated, such that expression, level, or activity is greater than or less than that observed in the absence of the modulator. For example, the term "modulate" can mean "inhibit," but the use of the word "modulate" is not limited to this definition.

[0108] By "inhibit" it is meant that the activity of a gene expression product or level of RNAs or equivalent RNAs encoding one or more gene products is reduced below that observed in the absence of the nucleic acid molecule of the invention. In one embodiment, inhibition with a siRNA molecule preferably is below that level observed in the presence of an inactive or attenuated molecule that is unable to mediate an RNAi response. In another embodiment, inhibition of gene expression with the siRNA molecule of the instant invention is greater in the presence of the siRNA molecule than in its absence.

[0109] By "gene" or "target gene" is meant, a nucleic acid that encodes an RNA, for example, nucleic acid sequences including, but not limited to, structural genes encoding a polypeptide. The target gene can be a gene derived from a cell, an endogenous gene, a transgene, or exogenous genes such as genes of a pathogen, for example a virus, which is present in the cell after infection thereof. The cell containing the target gene can be derived from or contained in any organism, for example a plant, animal, protozoan, virus, bacterium, or fungus. Non-limiting examples of plants include monocots, dicots, or gymnosperms. Non-limiting examples of animals include vertebrates or invertebrates. Non-limiting examples of fungi include molds or yeasts.

[0110] By "HIV" as used herein is meant, any virus, protein, peptide, polypeptide, and/or polynucleotide expressed from a HIV gene, for example entire viruses such as HIV-1, HIV-2, FIV-1, SIV-1 or viral components such as nef, vif, tat, or rev viral gene products.

[0111] By "highly conserved sequence region" is meant, a nucleotide sequence of one or more regions in a target gene does not vary significantly from one generation to the other or from one biological system to the other.

[0112] By "complementarity" or "complementary" is meant that a nucleic acid can form hydrogen bond(s) with another nucleic acid sequence by either traditional Watson-Crick or other non-traditional types of interaction. In reference to the nucleic molecules of the present invention, the binding free energy for a nucleic acid molecule with its complementary sequence is sufficient to allow the relevant function of the nucleic acid to proceed, e.g., RNAi activity. For example, the degree of complementarity between the sense and antisense strand of the siRNA construct can be the same or different from the degree of complementarity between the antisense strand of the siRNA and the target RNA sequence. Complementarity to the target sequence of less than 100% in the antisense strand of the siRNA duplex, including point mutations, is reported not to be tolerated when these changes are located between the 3'-end and the middle of the antisense siRNA (completely abolishes siRNA activity), whereas mutations near the 5 '-end of the antisense siRNA strand can exhibit a small degree of RNAi activity (Elbashir et al., 2001, *The EMBO Journal,* 20, 6877-6888). Determination of binding free energies for nucleic acid molecules is well known in the art (see, e.g., Turner et al., 1987, *CSH Symp. Quant. Biol.* LII pp.123-133; Frier et al., 1986, *Proc. Nat. Acad. Sci.* USA 83:9373-9377; Turner et al., 1987, *J Am. Chem. Soc.* 109:3783-3785). A percent complementarity indicates the percentage of contiguous residues in a nucleic acid molecule that can form hydrogen bonds (e.g., Watson-Crick base pairing) with a second nucleic acid sequence (e.g., 5, 6, 7, 8, 9, 10 out of 10 being 50%, 60%, 70%, 80%, 90%, and 100% complementary). "Perfectly complementary" means that all the contiguous residues of a nucleic acid sequence will hydrogen bond with the same number of contiguous residues in a second nucleic acid sequence.

[0113] The siRNA molecules of the invention represent a novel therapeutic approach to treat a variety of pathologic indications or other conditions, such as HIV infection or acquired immunodeficiency syndrome (AIDS) and any other diseases or conditions that are related to the levels of HIV in a cell or tissue, alone or in combination with other therapies. The reduction of HIV expression (specifically HIV RNA levels) and thus reduction in the level of the respective protein(s) relieves, to some extent, the symptoms of the disease or condition.

[0114] In one embodiment of the present invention, each sequence of a siRNA molecule of the invention is indepen-

dently about 18 to about 24 nucleotides in length, in specific embodiments about 18, 19, 20, 21, 22, 23, or 24 nucleotides in length. In another embodiment, the siRNA duplexes of the invention independently comprise between about 17 and about 23, for example, about 17, 18, 19, 20, 21, 22, or 23 base pairs. In yet another embodiment, siRNA molecules of the invention comprising hairpin or circular structures are about 35 to about 55, for example, about 35, 40, 45, 50 or 55 nucleotides in length, or about 38 to about 44, for example, about 38, 39, 40, 41, 42, 43 or 44 nucleotides in length and comprising about 16 to about 22, for example, about 16, 17, 18, 19, 20, 21 or 22 base pairs. Exemplary siRNA molecules of the invention are shown in Table I and/or **FIGS. 4 and 5**.

[**0115**] As used herein "cell" is used in its usual biological sense, and does not refer to an entire multicellular organism, e.g., specifically does not refer to a human. The cell can be present in an organism, e.g mammals such as humans, cows, sheep, apes, monkeys, swine, dogs, and cats. The cell can be eukaryotic (e.g., a mammalian cell). The cell can be of somatic or germ line origin, totipotent or pluripotent, dividing or non-dividing. The cell can also be derived from or can comprise a gamete or embryo, a stem cell, or a fully differentiated cell.

[**0116**] The siRNA molecules of the invention are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells or tissues. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In particular embodiments, the nucleic acid molecules of the invention comprise sequences shown in Table I and/or **FIGS. 4 and 5**. Examples of such nucleic acid molecules consist essentially of sequences defined in this table.

[**0117**] In another aspect, the invention provides mammalian cells containing one or more siRNA molecules of this invention. The one or more siRNA molecules can independently be targeted to the same or different sites.

[**0118**] By "RNA" is meant a molecule comprising at least one ribonucleotide residue. By "ribonucleotide" is meant a nucleotide with a hydroxyl group at the 2' position of a β-D-ribo-furanose moiety. The terms include double stranded RNA, single stranded RNA, isolated RNA such as partially purified RNA, essentially pure RNA, synthetic RNA, recombinantly produced RNA, as well as altered RNA that differs from naturally occurring RNA by the addition, deletion, substitution and/or alteration of one or more nucleotides. Such alterations can include addition of non-nucleotide material, such as to the end(s) of the siRNA or internally, for example at one or more nucleotides of the RNA. Nucleotides in the RNA molecules of the instant invention can also comprise non-standard nucleotides, such as non-naturally occurring nucleotides or chemically synthesized nucleotides or deoxynucleotides. These altered RNAs can be referred to as analogs or analogs of naturally-occurring RNA.

[**0119**] By "subject" is meant an organism, which is a donor or recipient of explanted cells or the cells themselves. "Subject" also refers to an organism to which the nucleic acid molecules of the invention can be administered. In one embodiment, a subject is a mammal or mammalian cells. In another embodiment, a subject is a human or human cells.

[**0120**] The term "phosphorothioate" as used herein refers to an internucleotide linkage having Formula I, wherein Z and/or W comprise a sulfur atom. Hence, the term phosphorothioate refers to both phosphorothioate and phosphorodithioate internucleotide linkages.

[**0121**] The term "universal base" as used herein refers to nucleotide base analogs that form base pairs with each of the natural DNA/RNA bases with little discrimination between them. Non-limiting examples of universal bases include C-phenyl, C-naphthyl and other aromatic derivatives, inosine, azole carboxamides, and nitroazole derivatives such as 3-nitropyrrole, 4-nitroindole, 5-nitroindole, and 6-nitroindole as known in the art (see for example Loakes, 2001, *Nucleic Acids Research,* 29, 2437-2447).

[**0122**] The term "acyclic nucleotide" as used herein refers to any nucleotide having an acyclic ribose sugar, for example where any of the ribose carbons (C1, C2, C3, C4, or C5), are independently or in combination absent from the nucleotide.

[**0123**] The nucleic acid molecules of the instant invention, individually, or in combination or in conjunction with other drugs, can be used to treat diseases or conditions discussed herein. For example, to treat a particular disease or condition, the siRNA molecules can be administered to a subject or can be administered to other appropriate cells evident to those skilled in the art, individually or in combination with one or more drugs under conditions suitable for the treatment.

[**0124**] In a further embodiment, the siRNA molecules can be used in combination with other known treatments to treat conditions or diseases discussed above. For example, the described molecules could be used in combination with one or more known therapeutic agents to treat a disease or condition. Non-limiting examples of other therapeutic agents that can be readily combined with a siRNA molecule of the invention are enzymatic nucleic acid molecules, allosteric nucleic acid molecules, antisense, decoy, or aptamer nucleic acid molecules, antibodies such as monoclonal antibodies, small molecules, and other organic and/or inorganic compounds including metals, salts and ions.

[**0125**] In one embodiment, the invention features an expression vector comprising a nucleic acid sequence encoding at least one siRNA molecule of the invention, in a manner which allows expression of the siRNA molecule. For example, the vector can contain sequence(s) encoding both strands of a siRNA molecule comprising a duplex. The vector can also contain sequence(s) encoding a single nucleic acid molecule that is self complimentary and thus forms a siRNA molecule. Non-limiting examples of such expression vectors are described in Paul et al., 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee et al., 2002, *Nature Biotechnology,* 19, 500; and Novina et al., 2002, *Nature Medicine,* advance online publication doi:10.1038/nm725.

[**0126**] In another embodiment, the invention features a mammalian cell, for example, a human cell, including an expression vector of the invention.

[**0127**] In yet another embodiment, the expression vector of the invention comprises a sequence for a siRNA molecule having complementarity to a RNA molecule referred to by a Genbank Accession numbers, for example HIV genes such

as Genbank Accession Nos. AJ302647 (HIV-1), NC_001722 (HIV-2), NC_001482 (FIV-1) and/or M66437 (SIV-1).

[0128] In one embodiment, an expression vector of the invention comprises a nucleic acid sequence encoding two or more siRNA molecules, which can be the same or different.

[0129] In another aspect of the invention, siRNA molecules that interact with target RNA molecules and down-regulate gene encoding target RNA molecules (for example target RNA molecules referred to by Genbank Accession numbers herein) are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siRNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siRNA molecules bind and down-regulate gene function or expression via RNA interference (RNAi). Delivery of siRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell.

[0130] By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

[0131] By "comprising" is meant including, but not limited to, whatever follows the word "comprising". Thus, use of the term "comprising" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present. By "consisting of" is meant including, and limited to, whatever follows the phrase "consisting of". Thus, the phrase "consisting of" indicates that the listed elements are required or mandatory, and that no other elements may be present. By "consisting essentially of" is meant including any elements listed after the phrase, and limited to other elements that do not interfere with or contribute to the activity or action specified in the disclosure for the listed elements. Thus, the phrase "consisting essentially of" indicates that the listed elements are required or mandatory, but that other elements are optional and may or may not be present depending upon whether or not they affect the activity or action of the listed elements.

[0132] Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0133] First the drawings will be described briefly.

[0134] Drawings

[0135] **FIG. 1** shows a non-limiting example of a scheme for the synthesis of siRNA molecules. The complimentary siRNA sequence strands, strand 1 and strand 2, are synthesized in tandem and are connected by a cleavable linkage, such as a nucleotide succinate or abasic succinate, which can be the same or different from the cleavable linker used for solid phase synthesis on a solid support. The synthesis can be either solid phase or solution phase, in the example shown, the synthesis is a solid phase synthesis. The synthesis is performed such that a protecting group, such as a dimethoxytrityl group, remains intact on the terminal nucleotide of the tandem oligonucleotide. Upon cleavage and deprotection of the oligonucleotide, the two siRNA strands spontaneously hybridize to form a siRNA duplex, which allows the purification of the duplex by utilizing the properties of the terminal protecting group, for example by applying a trityl on purification method wherein only duplexes/oligonucleotides with the terminal protecting group are isolated.

[0136] **FIG. 2** shows a MALDI-TOV mass spectrum of a purified siRNA duplex synthesized by a method of the invention. The two peaks shown correspond to the predicted mass of the separate siRNA sequence strands. This result demonstrates that the siRNA duplex generated from tandem synthesis can be purified as a single entity using a simple trityl-on purification methodology.

[0137] **FIG. 3** shows a non-limiting proposed mechanistic representation of target RNA degradation involved in RNAi. Double stranded RNA (dsRNA), which is generated by RNA dependent RNA polymerase (RdRP) from foreign single stranded RNA, for example viral, transposon, or other exogenous RNA, activates the DICER enzyme which in turn generates siRNA duplexes having terminal phosphate groups (P). An active siRNA complex forms which recognizes a target RNA, resulting in degradation of the target RNA by the RISC endonuclease complex or in the synthesis of additional RNA by RNA dependent RNA polymerase (RdRP), which can activate DICER and result in additional siRNA molecules, thereby amplifying the RNAi response.

[0138] **FIG. 4** shows non-limiting examples of chemically modified siRNA constructs of the present invention. In the figure, N stands for any nucleotide (adenosine, guanosine, cytosine, uridine, or optionally thymidine, for example thymidine can be substituted in the overhanging regions designated by parenthesis (N N). Various modifications are shown for the sense and antisense strands of the siRNA constructs. A The sense strand comprises 21 nucleotides having four phosphorothioate 5' and 3'-terminal internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and four 5'-terminal phosphorothioate internucleotide linkages and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. B The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl modified

nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. C The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl modified nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro modified nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. D The sense strand comprises 21 nucleotides having five phosphorothioate 5' and 3'-terminal internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all nucleotides are ribonucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and five 5'-terminal phosphorothioate internucleotide linkages and wherein all nucleotides are ribonucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. E The sense strand comprises 21 nucleotides wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides all having phosphorothioate internucleotide linkages, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and wherein all nucleotides are ribonucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. F The sense strand comprises 21 nucleotides having 5'- and 3'-terminal cap moieties, wherein the two terminal 3'-nucleotides are optionally base paired and wherein all pyrimidine nucleotides that may be present are 2'-O-methyl nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand comprises 21 nucleotides, wherein the two terminal 3'-nucleotides are optionally complimentary to the target RNA sequence, and having one 3'-terminal phosphorothioate internucleotide linkage and wherein all pyrimidine nucleotides that may be present are 2'-deoxy-2'-fluoro nucleotides except for (N N) nucleotides, which can comprise naturally occurring ribonucleotides, deoxynucleotides, universal bases, or other chemical modifications described herein. The antisense strand of constructs A-F comprise sequence complimentary to target RNA sequence of the invention.

[0139] **FIG. 5** shows non-limiting examples of specific chemically modified siRNA sequences of the invention. A-F applies the chemical modifications described in FIGS. **4**A-F to a HIV siRNA sequence.

[0140] **FIG. 6** shows non-limiting examples of different siRNA constructs of the invention. The examples shown (constructs 1, 2, and 3) have 19 representative base pairs, however, different embodiments of the invention include any number of base pairs described herein. Bracketed regions represent nucleotide overhangs, for example comprising between about 1, 2, 3, or 4 nucleotides in length, preferably about 2 nucleotides. Constructs 1 and 2 can be used independently for RNAi activity. Construct 2 can comprise a polynucleotide or non-nucleotide linker, which can optionally be designed as a biodegradable linker. In one embodiment, the loop structure shown in construct 2 can comprise a biodegradable linker that results in the formation of construct 1 in vivo and/or in vitro. In another example, construct 3 can be used to generate construct 2 under the same principle wherein a linker is used to generate the active siRNA construct 2 in vivo and/or in vitro, which can optionally utilize another biodegradable linker to generate the active siRNA construct 1 in vivo and/or in vitro. As such, the stability and/or activity of the siRNA constructs can be modulated based on the design of the siRNA construct for use in vivo or in vitro and/or in vitro.

[0141] **FIG. 7** is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate siRNA hairpin constructs. (A) A DNA oligomer is synthesized with a 5'-restriction site (R1) sequence followed by a region having sequence identical (sense region of siRNA) to a predetermined HIV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, which is followed by a loop sequence of defined sequence (X), comprising, for example, between about 3 and 10 nucleotides. (B) The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self complementary sequence that will result in a siRNA transcript having specificity for an HIV target sequence and having self complementary sense and antisense regions. (C) The construct is heated (for example to about 95° C.) to linearize the sequence, thus allowing extension of a complementary second DNA strand using a primer to the 3'-restriction sequence of the first strand. The double stranded DNA is then inserted into an appropriate vector for expression in cells. The construct can be designed such that a 3'-overhang results from the transcription, for example by engineering restriction sites and/or utilizing a poly-U termination region as described in Paul et al., 2002, *Nature Biotechnology,* 29, 505-508.

[0142] **FIG. 8** is a diagrammatic representation of a scheme utilized in generating an expression cassette to generate double stranded siRNA constructs. (A) A DNA oligomer is synthesized with a 5'-restriction (R1) site

sequence followed by a region having sequence identical (sense region of siRNA) to a predetermined HIV target sequence, wherein the sense region comprises, for example, about 19, 20, 21, or 22 nucleotides (N) in length, and which is followed by a 3'-restriction site (R2) which is adjacent to a loop sequence of defined sequence (X). (B) The synthetic construct is then extended by DNA polymerase to generate a hairpin structure having self complementary sequence. (C) The construct is processed by restriction enzymes specific to R1 and R2 to generate a double stranded DNA which is then inserted into an appropriate vector for expression in cells. The transcription cassette is designed such that a U6 promoter region flanks each side of the dsDNA which generates the separate sense and antisense strands of the siRNA. Poly T termination sequences can be added to the constructs to generate U overhangs in the resulting transcript.

[0143] **FIG. 9** is a diagrammatic representation of a method used to determine target sites for siRNA mediated RNAi within a particular target nucleic acid sequence, such as messenger RNA. (A) A pool of siRNA oligonucleotides are synthesized wherein the antisense region of the siRNA constructs has complementarity to target sites across the target nucleic acid sequence, and wherein the sense region comprises sequence complementary to the antisense region of the siRNA. (B) The sequences are pooled and are inserted into vectors such that (C) transfection of a vector into cells results in the expression of the siRNA. (D) Cells are sorted based on phenotypic change that is associated with modulation of the target nucleic acid sequence. (E) The siRNA is isolated from the sorted cells and is sequenced to identify efficacious target sites within the target nucleic acid sequence.

[0144] Mechanism of Action of Nucleic Acid Molecules of the Invention

[0145] RNA interference refers to the process of sequence specific post transcriptional gene silencing in animals mediated by short interfering RNAs (siRNA) (Fire et al., 1998, *Nature,* 391, 806). The corresponding process in plants is commonly referred to as post transcriptional gene silencing or RNA silencing and is also referred to as quelling in fungi. The process of post transcriptional gene silencing is thought to be an evolutionarily conserved cellular defense mechanism used to prevent the expression of foreign genes which is commonly shared by diverse flora and phyla (Fire et al., 1999, *Trends Genet.,* 15, 358). Such protection from foreign gene expression may have evolved in response to the production of double stranded RNAs (dsRNA) derived from viral infection or the random integration of transposon elements into a host genome via a cellular response that specifically destroys homologous single stranded RNA or viral genomic RNA. The presence of dsRNA in cells triggers the RNAi response though a mechanism that has yet to be fully characterized. This mechanism appears to be different from the interferon response that results from dsRNA mediated activation of protein kinase PKR and 2',5'-oligoadenylate synthetase resulting in non-specific cleavage of mRNA by ribonuclease L.

[0146] The presence of long dsRNAs in cells stimulates the activity of a ribonuclease III enzyme referred to as dicer. Dicer is involved in the processing of the dsRNA into short pieces of dsRNA known as short interfering RNAs (siRNA) (Berstein et al., 2001, *Nature,* 409, 363). Short interfering RNAs derived from dicer activity are typically about 21 to about 23 (i.e., about 21, 22 or 23) nucleotides in length and comprise about 19 base pair duplexes. Dicer has also been implicated in the excision of 21 and 22 nucleotide small temporal RNAs (stRNA) from precursor RNA of conserved structure that are implicated in translational control (Hutvagner et al., 2001, *Science,* 293, 834). The RNAi response also features an endonuclease complex containing a siRNA, commonly referred to as an RNA-induced silencing complex (RISC), which mediates cleavage of single stranded RNA having sequence homologous to the siRNA. Cleavage of the target RNA takes place in the middle of the region complementary to the guide sequence of the siRNA duplex (Elbashir et al., 2001, *Genes Dev.,* 15, 188).

[0147] Short interfering RNA mediated RNAi has been studied in a variety of systems. Fire et al., 1998, *Nature,* 391, 806, were the first to observe RNAi in *C. Elegans.* Wianny and Goetz, 1999, *Nature Cell Biol.,* 2, 70, describes RNAi mediated by dsRNA in mouse embryos. Hammond et al., 2000, *Nature,* 404, 293, describe RNAi in Drosophila cells transfected with dsRNA. Elbashir et al., 2001, *Nature,* 411, 494, describe RNAi induced by introduction of duplexes of synthetic 21-nucleotide RNAs in cultured mammalian cells including human embryonic kidney and HeLa cells. Recent work in Drosophila embryonic lysates has revealed certain requirements for siRNA length, structure, chemical composition, and sequence that are essential to mediate efficient RNAi activity. These studies have shown that 21 nucleotide siRNA duplexes are most active when containing two nucleotide 3'-overhangs. Furthermore, substitution of one or both siRNA strands with 2'-deoxy or 2'-O-methyl nucleotides abolishes RNAi activity, whereas substitution of 3'-terminal siRNA nucleotides with deoxy nucleotides was shown to be tolerated. Mismatch sequences in the center of the siRNA duplex were also shown to abolish RNAi activity. In addition, these studies also indicate that the position of the cleavage site in the target RNA is defined by the 5'-end of the siRNA guide sequence rather than the 3'-end (Elbashir et al., 2001, *EMBO J.,* 20, 6877). Other studies have indicated that a 5'-phosphate on the target-complementary strand of a siRNA duplex is required for siRNA activity and that ATP is utilized to maintain the 5'-phosphate moiety on the siRNA (Nykanen et al., 2001, *Cell,* 107, 309), however siRNA molecules lacking a 5'-phosphate are active when introduced exogenously, suggesting that 5'-phosphorylation of siRNA constructs may occur in vivo.

[0148] Synthesis of Nucleic Acid Molecules

[0149] Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small nucleic acid motifs ("small" refers to nucleic acid motifs no more than 100 nucleotides in length, preferably no more than 80 nucleotides in length, and most preferably no more than 50 nucleotides in length; e.g., individual siRNA oligonucleotide sequences or siRNA sequences synthesized in tandem) are preferably used for exogenous delivery. The simple structure of these molecules increases the ability of the nucleic acid to invade targeted regions of protein and/or RNA structure. Exemplary molecules of the instant invention are chemically synthesized, and others can similarly be synthesized.

[0150] Oligonucleotides (e.g., certain modified oligonucleotides or portions of oligonucleotides lacking ribo-

nucleotides) are synthesized using protocols known in the art, for example as described in Caruthers et al., 1992, *Methods in Enzymology* 211, 3-19, Thompson et al., International PCT Publication No. WO 99/54459, Wincott et al., 1995, *Nucleic Acids Res.* 23, 2677-2684, Wincott et al., 1997, *Methods Mol. Bio.,* 74, 59, Brennan et al., 1998, *Biotechnol Bioeng.,* 61, 33-45, and Brennan, U.S. Pat. No. 6,001,311. All of these references are incorporated herein by reference. The synthesis of oligonucleotides makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 2.5 min coupling step for 2'-O-methylated nucleotides and a 45 sec coupling step for 2'-deoxy nucleotides or 2'-deoxy-2'-fluoro nucleotides. Table II outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be performed on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M=6.6 μmol) of 2'-O-methyl phosphoramidite and a 105-fold excess of S-ethyl tetrazole (60 μL of 0.25 M=15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 22-fold excess (40 μL of 0.11 M=4.4 μmol) of deoxy phosphoramidite and a 70-fold excess of S-ethyl tetrazole (40 μL of 0.25 M=10 μmol) can be used in each coupling cycle of deoxy residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); and oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide, 0.05 M in acetonitrile) is used.

[0151] Deprotection of the DNA-based oligonucleotides is performed as follows: the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65° C. for 10 min. After cooling to −20° C., the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder.

[0152] The method of synthesis used for RNA including certain siRNA molecules of the invention follows the procedure as described in Usman et al., 1987, *J. Am. Chem. Soc.,* 109, 7845; Scaringe et al., 1990, *Nucleic Acids Res.,* 18, 5433; and Wincott et al., 1995, *Nucleic Acids Res.* 23, 2677-2684 Wincott et al., 1997, *Methods Mol. Bio.,* 74, 59, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. In a non-limiting example, small scale syntheses are conducted on a 394 Applied Biosystems, Inc. synthesizer using a 0.2 μmol scale protocol with a 7.5 min coupling step for alkylsilyl protected nucleotides and a 2.5 min coupling step for 2'-O-methylated nucleotides. Table II outlines the amounts and the contact times of the reagents used in the synthesis cycle. Alternatively, syntheses at the 0.2 μmol scale can be done on a 96-well plate synthesizer, such as the instrument produced by Protogene (Palo Alto, Calif.) with minimal modification to the cycle. A 33-fold excess (60 μL of 0.11 M=6.6 μmol) of 2'-O-methyl phosphoramidite and a 75-fold excess of S-ethyl tetrazole (60 μL of 0.25 M=15 μmol) can be used in each coupling cycle of 2'-O-methyl residues relative to polymer-bound 5'-hydroxyl. A 66-fold excess (120 μL of 0.11 M=13.2 μmol) of alkylsilyl (ribo) protected phosphoramidite and a 150-fold excess of S-ethyl tetrazole (120 μL of 0.25 M=30 μmol) can be used in each coupling cycle of ribo residues relative to polymer-bound 5'-hydroxyl. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, are typically 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer include the following: detritylation solution is 3% TCA in methylene chloride (ABI); capping is performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution is 16.9 mM I_2, 49 mM pyridine, 9% water in THF (PERSEPTIVE™). Burdick & Jackson Synthesis Grade acetonitrile is used directly from the reagent bottle. S-Ethyltetrazole solution (0.25 M in acetonitrile) is made up from the solid obtained from American International Chemical, Inc. Alternately, for the introduction of phosphorothioate linkages, Beaucage reagent (3H-1,2-Benzodithiol-3-one 1,1-dioxide 0.05 M in acetonitrile) is used.

[0153] Deprotection of the RNA is performed using either a two-pot or one-pot protocol. For the two-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 40% aq. methylamine (1 mL) at 65° C. for 10 min. After cooling to −20° C., the supernatant is removed from the polymer support. The support is washed three times with 1.0 mL of EtOH:MeCN:H2O/3:1:1, vortexed and the supernatant is then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, are dried to a white powder. The base deprotected oligoribonucleotide is resuspended in anhydrous TEA/HF/NMP solution (300 μL of a solution of 1.5 mL N-methylpyrrolidinone, 750 μL TEA and 1 mL TEA.3HF to provide a 1.4 M HF concentration) and heated to 65° C. After 1.5 h, the oligomer is quenched with 1.5 M NH_4HCO_3.

[0154] Alternatively, for the one-pot protocol, the polymer-bound trityl-on oligoribonucleotide is transferred to a 4 mL glass screw top vial and suspended in a solution of 33% ethanolic methylamine/DMSO: 1/1 (0.8 mL) at 65° C. for 15 min. The vial is brought to r.t. TEA.3HF (0.1 mL) is added and the vial is heated at 65° C. for 15 min. The sample is cooled at −20° C. and then quenched with 1.5 M NH_4HCO_3.

[0155] For purification of the trityl-on oligomers, the quenched NH_4HCO_3 solution is loaded onto a C-18 containing cartridge that had been prewashed with acetonitrile followed by 50 mM TEAA. After washing the loaded cartridge with water, the RNA is detritylated with 0.5% TFA

for 13 min. The cartridge is then washed again with water, salt exchanged with 1 M NaCl and washed with water again. The oligonucleotide is then eluted with 30% acetonitrile.

[0156] The average stepwise coupling yields are typically >98% (Wincott et al., 1995 *Nucleic Acids Res.* 23, 2677-2684). Those of ordinary skill in the art will recognize that the scale of synthesis can be adapted to be larger or smaller than the example described above including but not limited to 96-well format, all that is important is the ratio of chemicals used in the reaction.

[0157] Alternatively, the nucleic acid molecules of the present invention can be synthesized separately and joined together post-synthetically, for example, by ligation (Moore et al., 1992, *Science* 256, 9923; Draper et al., International PCT publication No. WO 93/23569; Shabarova et al., 1991, *Nucleic Acids Research* 19, 4247; Bellon et al., 1997, *Nucleosides & Nucleotides,* 16, 951; Bellon et al., 1997, *Bioconjugate Chem.* 8, 204), or by hybridization following synthesis and/or deprotection.

[0158] The siRNA molecules of the invention can also be synthesized via a tandem synthesis methodology as described in Example 1 herein, wherein both siRNA strands are synthesized as a contiguous oligonucleotide sequence separated by a cleavable linker which is subsequently cleaved to provide separate siRNA sequences that hybridize and permit purification of the siRNA duplex. The tandem synthesis of siRNA as described herein can be readily adapted to both multiwell/multiplate synthesis platforms such as 96 well or similarly larger multi-well platforms. The tandem synthesis of siRNA as described herein can also be readily adapted to large scale synthesis platforms employing batch reactors, synthesis columns and the like.

[0159] The nucleic acid molecules of the present invention can be modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992, *TIBS* 17, 34; Usman et al., 1994, *Nucleic Acids Symp. Ser.* 31, 163). siRNA constructs can be purified by gel electrophoresis using general methods or can be purified by high pressure liquid chromatography (HPLC; see Wincott et al., supra, the totality of which is hereby incorporated herein by reference) and re-suspended in water.

[0160] In another aspect of the invention, siRNA molecules of the invention are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. The recombinant vectors capable of expressing the siRNA molecules can be delivered as described herein, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of siRNA molecules.

[0161] Optimizing Activity of the Nucleic Acid Molecule of the Invention.

[0162] Chemically synthesizing nucleic acid molecules with modifications (base, sugar and/or phosphate) can prevent their degradation by serum ribonucleases, which can increase their potency (see e.g., Eckstein et al., International Publication No. WO 92/07065; Perrault et al., 1990 *Nature* 344, 565; Pieken et al., 1991, *Science* 253, 314; Usman and Cedergren, 1992, *Trends in Biochem. Sci.* 17, 334; Usman et al., International Publication No. WO 93/15187; and Rossi et al., International Publication No. WO 91/03162; Sproat, U.S. Pat. No. 5,334,711; Gold et al., U.S. Pat. No. 6,300,074; and Burgin et al., supra; all of which are incorporated by reference herein). All of the above references describe various chemical modifications that can be made to the base, phosphate and/or sugar moieties of the nucleic acid molecules described herein. Modifications that enhance their efficacy in cells, and removal of bases from nucleic acid molecules to shorten oligonucleotide synthesis times and reduce chemical requirements are desired.

[0163] There are several examples in the art describing sugar, base and phosphate modifications that can be introduced into nucleic acid molecules with significant enhancement in their nuclease stability and efficacy. For example, oligonucleotides are modified to enhance stability and/or enhance biological activity by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-flouro, 2'-O-methyl, 2'-O-allyl, 2'-H, nucleotide base modifications (for a review see Usman and Cedergren, 1992, *TIBS.* 17, 34; Usman et al., 1994, *Nucleic Acids Symp. Ser.* 31, 163; Burgin et al., 1996, *Biochemistry,* 35, 14090). Sugar modification of nucleic acid molecules have been extensively described in the art (see Eckstein et al., *International Publication* PCT No. WO 92/07065; Perrault et al. *Nature,* 1990, 344, 565-568; Pieken et al. *Science,* 1991, 253, 314-317; Usman and Cedergren, *Trends in Biochem. Sci.,* 1992, 17, 334-339; Usman et al. *International Publication* PCT No. WO 93/15187; Sproat, U.S. Pat. No. 5,334,711 and Beigelman et al., 1995, *J. Biol. Chem.,* 270, 25702; Beigelman et al., International PCT publication No. WO 97/26270; Beigelman et al., U.S. Pat. No. 5,716,824; Usman et al., U.S. Pat. No. 5,627,053; Woolf et al., International PCT Publication No. WO 98/13526; Thompson et al., U.S. Ser. No. 60/082,404 which was filed on Apr. 20, 1998; Karpeisky et al., 1998, *Tetrahedron Lett.,* 39, 1131; Earnshaw and Gait, 1998, *Biopolymers* (*Nucleic Acid Sciences*), 48, 39-55; Verma and Eckstein, 1998, *Annu. Rev. Biochem.,* 67, 99-134; and Burlina et al., 1997, *Bioorg. Med. Chem.,* 5, 1999-2010; all of the references are hereby incorporated in their totality by reference herein). Such publications describe general methods and strategies to determine the location of incorporation of sugar, base and/or phosphate modifications and the like into nucleic acid molecules without modulating catalysis, and are incorporated by reference herein. In view of such teachings, similar modifications can be used as described herein to modify the siRNA nucleic acid molecules of the instant invention so long as the ability of siRNA to promote RNAi is cells is not significantly inhibited.

[0164] While chemical modification of oligonucleotide internucleotide linkages with phosphorothioate, phosphorothioate, and/or 5'-methylphosphonate linkages improves stability, excessive modifications can cause some toxicity or decreased activity. Therefore, when designing nucleic acid molecules, the amount of these internucleotide linkages should be minimized. The reduction in the concentration of these linkages should lower toxicity, resulting in increased efficacy and higher specificity of these molecules.

[0165] Small interfering RNA (siRNA) molecules having chemical modifications that maintain or enhance activity are provided. Such a nucleic acid is also generally more resistant

to nucleases than an unmodified nucleic acid. Accordingly, the in vitro and/or in vivo activity should not be significantly lowered. In cases in which modulation is the goal, therapeutic nucleic acid molecules delivered exogenously should optimally be stable within cells until translation of the target RNA has been modulated long enough to reduce the levels of the undesirable protein. This period of time varies between hours to days depending upon the disease state. Improvements in the chemical synthesis of RNA and DNA (Wincott et al., 1995 *Nucleic Acids Res.* 23, 2677; Caruthers et al., 1992, *Methods in Enzymology* 211,3-19 (incorporated by reference herein)) have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability, as described above.

[**0166**] In one embodiment, nucleic acid molecules of the invention include one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more G-clamp nucleotides. A G-clamp nucleotide is a modified cytosine analog wherein the modifications confer the ability to hydrogen bond both Watson-Crick and Hoogsteen faces of a complementary guanine within a duplex, see for example Lin and Matteucci, 1998, *J. Am. Chem. Soc.,* 120, 8531-8532. A single G-clamp analog substitution within an oligonucleotide can result in substantially enhanced helical thermal stability and mismatch discrimination when hybridized to complementary oligonucleotides. The inclusion of such nucleotides in nucleic acid molecules of the invention results in both enhanced affinity and specificity to nucleic acid targets, complimentary sequences, or template strands. In another embodiment, nucleic acid molecules of the invention include one or more, for example, about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more LNA "locked nucleic acid" nucleotides such as a 2', 4'-C mythylene bicyclo nucleotide (see for example Wengel et al., International PCT Publication No. WO 00/66604 and WO 99/14226).

[**0167**] In another embodiment, the invention features conjugates and/or complexes of siRNA molecules of the invention. Such conjugates and/or complexes can be used to facilitate delivery of siRNA molecules into a biological system, such as a cell. The conjugates and complexes provided by the instant invention can impart therapeutic activity by transferring therapeutic compounds across cellular membranes, altering the pharmacokinetics, and/or modulating the localization of nucleic acid molecules of the invention. The present invention encompasses the design and synthesis of novel conjugates and complexes for the delivery of molecules, including, but not limited to, small molecules, lipids, phospholipids, nucleosides, nucleotides, nucleic acids, antibodies, toxins, negatively charged polymers and other polymers, for example proteins, peptides, hormones, carbohydrates, polyethylene glycols, or polyamines, across cellular membranes. In general, the transporters described are designed to be used either individually or as part of a multi-component system, with or without degradable linkers. These compounds are expected to improve delivery and/or localization of nucleic acid molecules of the invention into a number of cell types originating from different tissues, in the presence or absence of serum (see Sullenger and Cech, U.S. Pat. No. 5,854,038). Conjugates of the molecules described herein can be attached to biologically active molecules via linkers that are biodegradable, such as biodegradable nucleic acid linker molecules.

[**0168**] The term "biodegradable nucleic acid linker molecule" as used herein, refers to a nucleic acid molecule that is designed as a biodegradable linker to connect one molecule to another molecule, for example, a biologically active molecule. The stability of the biodegradable nucleic acid linker molecule can be modulated by using various combinations of ribonucleotides, deoxyribonucleotides, and chemically modified nucleotides, for example, 2'-O-methyl, 2'-fluoro, 2'-amino, 2'-O-amino, 2'-C-allyl, 2'-O-allyl, and other 2'-modified or base modified nucleotides. The biodegradable nucleic acid linker molecule can be a dimer, trimer, tetramer or longer nucleic acid molecule, for example, an oligonucleotide of about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 nucleotides in length, or can comprise a single nucleotide with a phosphorus-based linkage, for example, a phosphoramidate or phosphodiester linkage. The biodegradable nucleic acid linker molecule can also comprise nucleic acid backbone, nucleic acid sugar, or nucleic acid base modifications.

[**0169**] The term "biodegradable" as used herein, refers to degradation in a biological system, for example enzymatic degradation or chemical degradation.

[**0170**] The term "biologically active molecule" as used herein, refers to compounds or molecules that are capable of eliciting or modifying a biological response in a system. Non-limiting examples of biologically active siRNA molecules either alone or in combination with othe molecules contemplated by the instant invention include therapeutically active molecules such as antibodies, hormones, antivirals, peptides, proteins, chemotherapeutics, small molecules, vitamins, co-factors, nucleosides, nucleotides, oligonucleotides, enzymatic nucleic acids, antisense nucleic acids, triplex forming oligonucleotides, 2,5-A chimeras, siRNA, dsRNA, allozymes, aptamers, decoys and analogs thereof. Biologically active molecules of the invention also include molecules capable of modulating the pharmacokinetics and/or pharmacodynamics of other biologically active molecules, for example, lipids and polymers such as polyamines, polyamides, polyethylene glycol and other polyethers.

[**0171**] The term "phospholipid" as used herein, refers to a hydrophobic molecule comprising at least one phosphorus group. For example, a phospholipid can comprise a phosphorus-containing group and saturated or unsaturated alkyl group, optionally substituted with OH, COOH, oxo, amine, or substituted or unsubstituted aryl groups.

[**0172**] Therapeutic nucleic acid molecules (e.g., siRNA molecules) delivered exogenously optimally are stable within cells until reverse trascription of the RNA has been modulated long enough to reduce the levels of the RNA transcript. The nucleic acid molecules are resistant to nucleases in order to function as effective intracellular therapeutic agents. Improvements in the chemical synthesis of nucleic acid molecules described in the instant invention and in the art have expanded the ability to modify nucleic acid molecules by introducing nucleotide modifications to enhance their nuclease stability as described above.

[**0173**] In yet another embodiment, siRNA molecules having chemical modifications that maintain or enhance enzymatic activity of proteins involved in RNAi are provided. Such nucleic acids are also generally more resistant to

nucleases than unmodified nucleic acids. Thus, in vitro and/or in vivo the activity should not be significantly lowered.

[0174] Use of the nucleic acid-based molecules of the invention will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siRNA molecules targeted to different genes; nucleic acid molecules coupled with known small molecule modulators; or intermittent treatment with combinations of molecules, including different motifs and/or other chemical or biological molecules). The treatment of subjects with siRNA molecules can also include combinations of different types of nucleic acid molecules, such as enzymatic nucleic acid molecules (ribozymes), allozymes, antisense, 2,5-A oligoadenylate, decoys, aptamers etc.

[0175] In another aspect a siRNA molecule of the invention comprises one or more 5' and/or a 3'-cap structure, for example on only the sense siRNA strand, antisense siRNA strand, or both siRNA strands.

[0176] By "cap structure" is meant chemical modifications, which have been incorporated at either terminus of the oligonucleotide (see, for example, Adamic et al., U.S. Pat. No. 5,998,203, incorporated by reference herein). These terminal modifications protect the nucleic acid molecule from exonuclease degradation, and can help in delivery and/or localization within a cell. The cap can be present at the 5'-terminus (5'-cap) or at the 3'-terminal (3'-cap) or can be present on both termini. In non-limiting examples: the 5'-cap is selected from the group comprising inverted abasic residue (moiety); 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide, 4'-thio nucleotide; carbocyclic nucleotide; 1,5-anhydrohexitol nucleotide; L-nucleotides; alpha-nucleotides; modified base nucleotide; phosphorodithioate linkage; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; acyclic 3,4-dihydroxybutyl nucleotide; acyclic 3,5-dihydroxypentyl nucleotide, 3'-3'-inverted nucleotide moiety; 3'-3'-inverted abasic moiety; 3'-2'-inverted nucleotide moiety; 3'-2'-inverted abasic moiety; 1,4-butanediol phosphate; 3'-phosphoramidate; hexylphosphate; aminohexyl phosphate; 3'-phosphate; 3'-phosphorothioate; phosphorodithioate; or bridging or non-bridging methylphosphonate moiety.

[0177] In yet another preferred embodiment, the 3'-cap is selected from a group comprising, 4',5'-methylene nucleotide; 1-(beta-D-erythrofuranosyl) nucleotide; 4'-thio nucleotide, carbocyclic nucleotide; 5'-amino-alkyl phosphate; 1,3-diamino-2-propyl phosphate; 3-aminopropyl phosphate; 6-aminohexyl phosphate; 1,2-aminododecyl phosphate; hydroxypropyl phosphate; 1,5-anhydrohexitol nucleotide; L-nucleotide; alpha-nucleotide; modified base nucleotide; phosphorodithioate; threo-pentofuranosyl nucleotide; acyclic 3',4'-seco nucleotide; 3,4-dihydroxybutyl nucleotide; 3,5-dihydroxypentyl nucleotide, 5'-5'-inverted nucleotide moiety; 5'-5'-inverted abasic moiety; 5'-phosphoramidate; 5'-phosphorothioate; 1,4-butanediol phosphate; 5'-amino; bridging and/or non-bridging 5'-phosphoramidate, phosphorothioate and/or phosphorodithioate, bridging or non bridging methylphosphonate and 5'-mercapto moieties (for more details see Beaucage and Iyer, 1993, *Tetrahedron* 49, 1925; incorporated by reference herein).

[0178] By the term "non-nucleotide" is meant any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is abasic in that it does not contain a commonly recognized nucleotide base, such as adenosine, guanine, cytosine, uracil or thymine and therefore lacks a base at the 1'-position.

[0179] An "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably, it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group can be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino, or SH. The term also includes alkenyl groups that are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably, it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2, halogen, $N(CH_3)_2$, amino, or SH. The term "alkyl" also includes alkynyl groups that have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably, it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO_2 or $N(CH_3)_2$, amino or SH.

[0180] Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group that has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an —C(O)—NH—R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an —C(O)—OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

[0181] By "nucleotide" as used herein is as recognized in the art to include natural bases (standard), and modified bases well known in the art. Such bases are generally located at the 1' position of a nucleotide sugar moiety. Nucleotides generally comprise a base, sugar and a phosphate group. The nucleotides can be unmodified or modified at the sugar, phosphate and/or base moiety, (also referred to interchange-

ably as nucleotide analogs, modified nucleotides, non-natural nucleotides, non-standard nucleotides and other; see, for example, Usman and McSwiggen, supra; Eckstein et al., International PCT Publication No. WO 92/07065; Usman et al., International PCT Publication No. WO 93/15187; Uhlman & Peyman, supra, all are hereby incorporated by reference herein). There are several examples of modified nucleic acid bases known in the art as summarized by Limbach et al., 1994, *Nucleic Acids Res.* 22, 2183. Some of the non-limiting examples of base modifications that can be introduced into nucleic acid molecules include, inosine, purine, pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyl uracil, dihydrouridine, naphthyl, aminophenyl, 5-alkylcytidines (e.g., 5-methylcytidine), 5-alkyluridines (e.g., ribothymidine), 5-halouridine (e.g., 5-bromouridine) or 6-azapyrimidines or 6-alkylpyrimidines (e.g. 6-methyluridine), propyne, and others (Burgin et al., 1996, *Biochemistry,* 35, 14090; Uhlman & Peyman, supra). By "modified bases" in this aspect is meant nucleotide bases other than adenine, guanine, cytosine and uracil at 1' position or their equivalents.

[0182] In one embodiment, the invention features modified siRNA molecules, with phosphate backbone modifications comprising one or more phosphorothioate, phosphorodithioate, methylphosphonate, phosphotriester, morpholino, amidate carbamate, carboxymethyl, acetamidate, polyamide, sulfonate, sulfonamide, sulfamate, formacetal, thioformacetal, and/or alkylsilyl, substitutions. For a review of oligonucleotide backbone modifications, see Hunziker and Leumann, 1995, *Nucleic Acid Analogues: Synthesis and Properties,* in *Modern Synthetic Methods,* VCH, 331-417, and Mesmaeker et al., 1994, *Novel Backbone Replacements for Oligonucleotides, in Carbohydrate Modifications in Antisense Research,* ACS, 24-39.

[0183] By "abasic" is meant sugar moieties lacking a base or having other chemical groups in place of a base at the 1' position, see for example Adamic et al., U.S. Pat. No. 5,998,203.

[0184] By "unmodified nucleoside" is meant one of the bases adenine, cytosine, guanine, thymine, uracil joined to the 1' carbon of β-D-ribo-furanose.

[0185] By "modified nucleoside" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base, sugar and/or phosphate.

[0186] In connection with 2'-modified nucleotides as described for the present invention, by "amino" is meant 2'-NH_2 or 2'-O—NH_2, which may be modified or unmodified. Such modified groups are described, for example, in Eckstein et al., U.S. Pat. No. 5,672,695 and Matulic-Adamic et al., U.S. Pat. No. 6,248,878, which are both incorporated by reference in their entireties.

[0187] Various modifications to nucleic acid siRNA structure can be made to enhance the utility of these molecules. Such modifications will enhance shelf-life, half-life in vitro, stability, and ease of introduction of such oligonucleotides to the target site, e.g., to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

[0188] Administration of Nucleic Acid Molecules

[0189] A siRNA molecule of the invention can be adapted for use to treat, for example conditions related to HIV infection and/or AIDS, alone or in combination with other therapies. For example, a siRNA molecule can comprise a delivery vehicle, including liposomes, for administration to a subject, carriers and diluents and their salts, and/or can be present in pharmaceutically acceptable formulations. Methods for the delivery of nucleic acid molecules are described in Akhtar et al., 1992, *Trends Cell Bio.,* 2, 139; *Delivery Strategies for Antisense Oligonucleotide Therapeutics,* ed. Akhtar, 1995, Maurer et al., 1999, *Mol. Membr. Biol.,* 16, 129-140; Hofland and Huang, 1999, *Handb. Exp. Pharmacol.,* 137, 165-192; and Lee et al., 2000, *ACS Symp. Ser.,* 752, 184-192, all of which are incorporated herein by reference. Beigelman et al., U.S. Pat. No. 6,395,713 and Sullivan et al., PCT WO 94/02595, further describes the general methods for delivery of nucleic acid molecules. Delivery of nucleic acid molecules of the invention to hematopoietic cells, such as T-cells, can be accomplished as is known in the art, see for example Draper, U.S. Pat. No. 6,622,854; Phillips et al., 1996, *Nature Medicine,* 2(10), 1154-1156; Smith et al., 1996, *Antiviral Research,* 32(2), 99-115; and Rudoll et al., 1996, *Gene Therapy,* 3(8), 695-705.

[0190] These protocols can be utilized for the delivery of virtually any nucleic acid molecule. Nucleic acid molecules can be administered to cells by a variety of methods known to those of skill in the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres, or by proteinaceous vectors (O'Hare and Normand, International PCT Publication No. WO 00/53722). Alternatively, the nucleic acid/vehicle combination is locally delivered by direct injection or by use of an infusion pump. Direct injection of the nucleic acid molecules of the invention, whether subcutaneous, intramuscular, or intradermal, can take place using standard needle and syringe methodologies, or by needle-free technologies such as those described in Conry et al., 1999, *Clin. Cancer Res.,* 5, 2330-2337 and Barry et al., International PCT Publication No. WO 99/31262. The molecules of the instant invention can be used as pharmaceutical agents. Pharmaceutical agents prevent, modulate the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state in a subject.

[0191] Thus, the invention features a pharmaceutical composition comprising one or more nucleic acid(s) of the invention in an acceptable carrier, such as a stabilizer, buffer, and the like. The polynucleotides of the invention can be administered (e.g., RNA, DNA or protein) and introduced into a subject by any standard means, with or without stabilizers, buffers, and the like, to form a pharmaceutical composition. When it is desired to use a liposome delivery mechanism, standard protocols for formation of liposomes can be followed. The compositions of the present invention can also be formulated and used as tablets, capsules or elixirs for oral administration, suppositories for rectal administration, sterile solutions, suspensions for injectable administration, and the other compositions known in the art.

[0192] The present invention also includes pharmaceutically acceptable formulations of the compounds described. These formulations include salts of the above compounds, e.g., acid addition salts, for example, salts of hydrochloric, hydrobromic, acetic acid, and benzene sulfonic acid.

[0193] A pharmacological composition or formulation refers to a composition or formulation in a form suitable for administration, e.g., systemic administration, into a cell or subject, including for example a human. Suitable forms, in part, depend upon the use or the route of entry, for example oral, transdermal, or by injection. Such forms should not prevent the composition or formulation from reaching a target cell (i.e., a cell to which the negatively charged nucleic acid is desirable for delivery). For example, pharmacological compositions injected into the blood stream should be soluble. Other factors are known in the art, and include considerations such as toxicity and forms that prevent the composition or formulation from exerting its effect.

[0194] By "systemic administration" is meant in vivo systemic absorption or accumulation of drugs in the blood stream followed by distribution throughout the entire body. Administration routes which lead to systemic absorption include, without limitation: intravenous, subcutaneous, intraperitoneal, inhalation, oral, intrapulmonary and intramuscular. Each of these administration routes expose the siRNA molecules of the invention to an accessible diseased tissue. The rate of entry of a drug into the circulation has been shown to be a function of molecular weight or size. The use of a liposome or other drug carrier comprising the compounds of the instant invention can potentially localize the drug, for example, in certain tissue types, such as the tissues of the reticular endothelial system (RES). A liposome formulation that can facilitate the association of drug with the surface of cells, such as, lymphocytes and macrophages is also useful. This approach can provide enhanced delivery of the drug to target cells by taking advantage of the specificity of macrophage and lymphocyte immune recognition of abnormal cells, such as cancer cells.

[0195] By "pharmaceutically acceptable formulation" is meant, a composition or formulation that allows for the effective distribution of the nucleic acid molecules of the instant invention in the physical location most suitable for their desired activity. Non-limiting examples of agents suitable for formulation with the nucleic acid molecules of the instant invention include: P-glycoprotein inhibitors (such as Pluronic P85), which can enhance entry of drugs into the CNS (Jolliet-Riant and Tillement, 1999, *Fundam. Clin. Pharmacol.,* 13, 16-26); biodegradable polymers, such as poly (DL-lactide-coglycolide) microspheres for sustained release delivery after intracerebral implantation (Emerich, DF et al, 1999, *Cell Transplant,* 8, 47-58) (Alkermes, Inc. Cambridge, Mass.); and loaded nanoparticles, such as those made of polybutylcyanoacrylate, which can deliver drugs across the blood brain barrier and can alter neuronal uptake mechanisms (*Prog Neuropsychopharmacol Biol Psychiatry,* 23, 941-949, 1999). Other non-limiting examples of delivery strategies for the nucleic acid molecules of the instant invention include material described in Boado et al., 1998, *J. Pharm. Sci.,* 87, 1308-1315; Tyler et al., 1999, *FEBS Lett.,* 421, 280-284; Pardridge et al., 1995, *PNAS USA.,* 92, 5592-5596; Boado, 1995, *Adv. Drug Delivery Rev.,* 15, 73-107; Aldrian-Herrada et al., 1998, *Nucleic Acids Res.,* 26, 4910-4916; and Tyler et al., 1999, *PNAS USA.,* 96, 7053-7058.

[0196] The invention also features the use of the composition comprising surface-modified liposomes containing poly (ethylene glycol) lipids (PEG-modified, or long-circulating liposomes or stealth liposomes). These formulations offer a method for increasing the accumulation of drugs in target tissues. This class of drug carriers resists opsonization and elimination by the mononuclear phagocytic system (MPS or RES), thereby enabling longer blood circulation times and enhanced tissue exposure for the encapsulated drug (Lasic et al. *Chem. Rev.* 1995, 95, 2601-2627; Ishiwata et al., *Chem. Pharm. Bull.* 1995, 43, 1005-1011). Such liposomes have been shown to accumulate selectively in tumors, presumably by extravasation and capture in the neovascularized target tissues (Lasic et al., *Science* 1995, 267, 1275-1276; Oku et al., 1995, *Biochim. Biophys. Acta,* 1238, 86-90). The long-circulating liposomes enhance the pharmacokinetics and pharmacodynamics of DNA and RNA, particularly compared to conventional cationic liposomes which are known to accumulate in tissues of the MPS (Liu et al., *J. Biol. Chem.* 1995, 42, 24864-24870; Choi et al., International PCT Publication No. WO 96/10391; Ansell et al., International PCT Publication No. WO 96/10390; Holland et al., International PCT Publication No. WO 96/10392). Long-circulating liposomes are also likely to protect drugs from nuclease degradation to a greater extent compared to cationic liposomes, based on their ability to avoid accumulation in metabolically aggressive MPS tissues such as the liver and spleen.

[0197] The present invention also includes compositions prepared for storage or administration, which include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences,* Mack Publishing Co. (A. R. Gennaro edit. 1985) hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

[0198] The present invention also includes compositions prepared for storage or administration that include a pharmaceutically effective amount of the desired compounds in a pharmaceutically acceptable carrier or diluent. Acceptable carriers or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in *Remington's Pharmaceutical Sciences,* Mack Publishing Co. (A. R. Gennaro edit. 1985), hereby incorporated by reference herein. For example, preservatives, stabilizers, dyes and flavoring agents can be provided. These include sodium benzoate, sorbic acid and esters of p-hydroxybenzoic acid. In addition, antioxidants and suspending agents can be used.

[0199] A pharmaceutically effective dose is that dose required to prevent, inhibit the occurrence, or treat (alleviate a symptom to some extent, preferably all of the symptoms) of a disease state. The pharmaceutically effective dose depends on the type of disease, the composition used, the route of administration, the type of mammal being treated, the physical characteristics of the specific mammal under consideration, concurrent medication, and other factors that those skilled in the medical arts will recognize. Generally, an amount between 0.1 mg/kg and 100 mg/kg body weight/day of active ingredients is administered dependent upon potency of the negatively charged polymer.

[0200] The nucleic acid molecules of the invention and formulations thereof can be administered orally, topically,

parenterally, by inhalation or spray, or rectally in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, adjuvants and/or vehicles. The term parenteral as used herein includes percutaneous, subcutaneous, intravascular (e.g., intravenous), intramuscular, or intrathecal injection or infusion techniques and the like. In addition, there is provided a pharmaceutical formulation comprising a nucleic acid molecule of the invention and a pharmaceutically acceptable carrier. One or more nucleic acid molecules of the invention can be present in association with one or more non-toxic pharmaceutically acceptable carriers and/or diluents and/or adjuvants, and if desired other active ingredients. The pharmaceutical compositions containing nucleic acid molecules of the invention can be in a form suitable for oral use, for example, as tablets, troches, lozenges, aqueous or oily suspensions, dispersible powders or granules, emulsion, hard or soft capsules, or syrups or elixirs.

[0201] Compositions intended for oral use can be prepared according to any method known to the art for the manufacture of pharmaceutical compositions and such compositions can contain one or more such sweetening agents, flavoring agents, coloring agents or preservative agents in order to provide pharmaceutically elegant and palatable preparations. Tablets contain the active ingredient in admixture with non-toxic pharmaceutically acceptable excipients that are suitable for the manufacture of tablets. These excipients can be, for example, inert diluents; such as calcium carbonate, sodium carbonate, lactose, calcium phosphate or sodium phosphate; granulating and disintegrating agents, for example, corn starch, or alginic acid; binding agents, for example starch, gelatin or acacia; and lubricating agents, for example magnesium stearate, stearic acid or talc. The tablets can be uncoated or they can be coated by known techniques. In some cases such coatings can be prepared by known techniques to delay disintegration and absorption in the gastrointestinal tract and thereby provide a sustained action over a longer period. For example, a time delay material such as glyceryl monosterate or glyceryl distearate can be employed.

[0202] Formulations for oral use can also be presented as hard gelatin capsules wherein the active ingredient is mixed with an inert solid diluent, for example, calcium carbonate, calcium phosphate or kaolin, or as soft gelatin capsules wherein the active ingredient is mixed with water or an oil medium, for example peanut oil, liquid paraffin or olive oil.

[0203] Aqueous suspensions contain the active materials in admixture with excipients suitable for the manufacture of aqueous suspensions. Such excipients are suspending agents, for example sodium carboxymethylcellulose, methylcellulose, hydropropyl-methylcellulose, sodium alginate, polyvinylpyrrolidone, gum tragacanth and gum acacia; dispersing or wetting agents can be a naturally-occurring phosphatide, for example, lecithin, or condensation products of an alkylene oxide with fatty acids, for example polyoxyethylene stearate, or condensation products of ethylene oxide with long chain aliphatic alcohols, for example heptadecaethyleneoxycetanol, or condensation products of ethylene oxide with partial esters derived from fatty acids and a hexitol such as polyoxyethylene sorbitol monooleate, or condensation products of ethylene oxide with partial esters derived from fatty acids and hexitol anhydrides, for example polyethylene sorbitan monooleate. The aqueous suspensions can also contain one or more preservatives, for example ethyl, or n-propyl p-hydroxybenzoate, one or more coloring agents, one or more flavoring agents, and one or more sweetening agents, such as sucrose or saccharin.

[0204] Oily suspensions can be formulated by suspending the active ingredients in a vegetable oil, for example arachis oil, olive oil, sesame oil or coconut oil, or in a mineral oil such as liquid paraffin. The oily suspensions can contain a thickening agent, for example beeswax, hard paraffin or cetyl alcohol. Sweetening agents and flavoring agents can be added to provide palatable oral preparations. These compositions can be preserved by the addition of an anti-oxidant such as ascorbic acid.

[0205] Dispersible powders and granules suitable for preparation of an aqueous suspension by the addition of water provide the active ingredient in admixture with a dispersing or wetting agent, suspending agent and one or more preservatives. Suitable dispersing or wetting agents or suspending agents are exemplified by those already mentioned above. Additional excipients, for example sweetening, flavoring and coloring agents, can also be present.

[0206] Pharmaceutical compositions of the invention can also be in the form of oil-in-water emulsions. The oily phase can be a vegetable oil or a mineral oil or mixtures of these. Suitable emulsifying agents can be naturally-occurring gums, for example gum acacia or gum tragacanth, naturally-occurring phosphatides, for example soy bean, lecithin, and esters or partial esters derived from fatty acids and hexitol, anhydrides, for example sorbitan monooleate, and condensation products of the said partial esters with ethylene oxide, for example polyoxyethylene sorbitan monooleate. The emulsions can also contain sweetening and flavoring agents.

[0207] Syrups and elixirs can be formulated with sweetening agents, for example glycerol, propylene glycol, sorbitol, glucose or sucrose. Such formulations can also contain a demulcent, a preservative and flavoring and coloring agents. The pharmaceutical compositions can be in the form of a sterile injectable aqueous or oleaginous suspension. This suspension can be formulated according to the known art using those suitable dispersing or wetting agents and suspending agents that have been mentioned above. The sterele injectable preparation can also be a sterile injectable solution or suspension in a non-toxic parentally acceptable diluent or solvent, for example as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that can be employed are water, Ringer's solution and isotonic sodium chloride solution. In addition, sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. In addition, fatty acids such as oleic acid find use in the preparation of injectables.

[0208] The nucleic acid molecules of the invention can also be administered in the form of suppositories, e.g., for rectal administration of the drug. These compositions can be prepared by mixing the drug with a suitable non-irritating excipient that is solid at ordinary temperatures but liquid at the rectal temperature and will therefore melt in the rectum to release the drug. Such materials include cocoa butter and polyethylene glycols.

[0209] Nucleic acid molecules of the invention can be administered parenterally in a sterile medium. The drug,

depending on the vehicle and concentration used, can either be suspended or dissolved in the vehicle. Advantageously, adjuvants such as local anesthetics, preservatives and buffering agents can be dissolved in the vehicle.

[0210] Dosage levels of the order of from about 0.1 mg to about 140 mg per kilogram of body weight per day are useful in the treatment of the above-indicated conditions (about 0.5 mg to about 7 g per subject per day). The amount of active ingredient that can be combined with the carrier materials to produce a single dosage form varies depending upon the host treated and the particular mode of administration. Dosage unit forms generally contain between from about 1 mg to about 500 mg of an active ingredient.

[0211] It is understood that the specific dose level for any particular subject depends upon a variety of factors including the activity of the specific compound employed, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[0212] For administration to non-human animals, the composition can also be added to the animal feed or drinking water. It can be convenient to formulate the animal feed and drinking water compositions so that the animal takes in a therapeutically appropriate quantity of the composition along with its diet. It can also be convenient to present the composition as a premix for addition to the feed or drinking water.

[0213] The nucleic acid molecules of the present invention can also be administered to a subject in combination with other therapeutic compounds to increase the overall therapeutic effect. The use of multiple compounds to treat an indication can increase the beneficial effects while reducing the presence of side effects.

[0214] In one embodiment, the invention compositions suitable for administering nucleic acid molecules of the invention to specific cell types, such as hepatocytes. For example, the asialoglycoprotein receptor (ASGPr) (Wu and Wu, 1987, *J. Biol. Chem.* 262, 4429-4432) is unique to hepatocytes and binds branched galactose-terminal glycoproteins, such as asialoorosomucoid (ASOR). Binding of such glycoproteins or synthetic glycoconjugates to the receptor takes place with an affinity that strongly depends on the degree of branching of the oligosaccharide chain, for example, triatennary structures are bound with greater affinity than biatenarry or monoatennary chains (Baenziger and Fiete, 1980, *Cell,* 22, 611-620; Connolly et al., 1982, *J. Biol. Chem.,* 257, 939-945). Lee and Lee, 1987, *Glycoconjugate J.,* 4, 317-328, obtained this high specificity through the use of N-acetyl-D-galactosamine as the carbohydrate moiety, which has higher affinity for the receptor, compared to galactose. This "clustering effect" has also been described for the binding and uptake of mannosyl-terminating glycoproteins or glycoconjugates (Ponpipom et al., 1981, *J. Med. Chem.,* 24, 1388-1395). The use of galactose and galactosamine based conjugates to transport exogenous compounds across cell membranes can provide a targeted delivery approach to the treatment of liver disease such as HBV infection or hepatocellular carcinoma. The use of bioconjugates can also provide a reduction in the required dose of therapeutic compounds required for treatment. Furthermore, therapeutic bioavialability, pharmacodynamics, and pharmacokinetic parameters can be modulated through the use of nucleic acid bioconjugates of the invention.

[0215] Alternatively, certain siRNA molecules of the instant invention can be expressed within cells from eukaryotic promoters (e.g., Izant and Weintraub, 1985, *Science,* 229, 345; McGarry and Lindquist, 1986, *Proc. Natl. Acad. Sci.,* USA 83, 399; Scanlon et al., 1991, *Proc. Natl. Acad. Sci. USA,* 88, 10591-5; Kashani-Sabet et al., 1992, *Antisense Res. Dev.,* 2, 3-15; Dropulic et al., 1992, *J. Virol.,* 66, 1432-41; Weerasinghe et al., 1991, *J. Virol.,* 65, 5531-4; Ojwang et al., 1992, *Proc. Natl. Acad. Sci. USA,* 89, 10802-6; Chen et al., 1992, *Nucleic Acids Res.,* 20, 4581-9; Sarver et al., 1990 *Science,* 247, 1222-1225; Thompson et al., 1995, *Nucleic Acids Res.,* 23, 2259; Good et al., 1997, *Gene Therapy,* 4, 45. Those skilled in the art realize that any nucleic acid can be expressed in eukaryotic cells from the appropriate DNA/RNA vector. The activity of such nucleic acids can be augmented by their release from the primary transcript by a enzymatic nucleic acid (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992, *Nucleic Acids Symp. Ser.,* 27, 15-6; Taira et al., 1991, *Nucleic Acids Res.,* 19, 5125-30; Ventura et al., 1993, *Nucleic Acids Res.,* 21, 3249-55; Chowrira et al., 1994, *J. Biol. Chem.,* 269, 25856.

[0216] In another aspect of the invention, RNA molecules of the present invention can be expressed from transcription units (see for example Couture et al., 1996, *TIG.,* 12, 510) inserted into DNA or RNA vectors. The recombinant vectors can be DNA plasmids or viral vectors. siRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. In another embodiment, pol III based constructs are used to express nucleic acid molecules of the invention (see for example Thompson, U.S. Pat. Nos. 5,902,880 and 6,146, 886). The recombinant vectors capable of expressing the siRNA molecules can be delivered as described above, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of nucleic acid molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the siRNA molecule interacts with the target mRNA and generates an RNAi response. Delivery of siRNA molecule expressing vectors can be systemic, such as by intravenous or intra-muscular administration, by administration to target cells ex-planted from a subject followed by reintroduction into the subject, or by any other means that would allow for introduction into the desired target cell (for a review see Couture et al., 1996, *TIG.,* 12, 510).

[0217] In one aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one siRNA molecule of the instant invention. The expression vector can encode one or both strands of a siRNA duplex, or a single self complimentary strand that self hybridizes into a siRNA duplex. The nucleic acid sequences encoding the siRNA molecules of the instant invention can be operably linked in a manner that allows expression of the siRNA molecule (see for example Paul et al., 2002, *Nature Biotechnology,* 19, 505; Miyagishi and Taira, 2002, *Nature Biotechnology,* 19, 497; Lee et al., 2002, *Nature Biotechnology,* 19, 500; and Novina et al., 2002, *Nature Medicine,* advance online publication doi:10.1038/nm725).

[0218] In another aspect, the invention features an expression vector comprising: a) a transcription initiation region

(e.g., eukaryotic pol I, II or III initiation region); b) a transcription termination region (e.g., eukaryotic pol I, II or III termination region); and c) a nucleic acid sequence encoding at least one of the siRNA molecules of the instant invention; wherein said sequence is operably linked to said initiation region and said termination region, in a manner that allows expression and/or delivery of the siRNA molecule. The vector can optionally include an open reading frame (ORF) for a protein operably linked on the 5' side or the 3'-side of the sequence encoding the siRNA of the invention; and/or an intron (intervening sequences).

[0219] Transcription of the siRNA molecule sequences can be driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters are expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type depends on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990, *Proc. Natl. Acad. Sci. U S A,* 87, 6743-7; Gao and Huang 1993, *Nucleic Acids Res.,* 21, 2867-72; Lieber et al., 1993, *Methods Enzymol.,* 217, 47-66; Zhou et al., 1990, *Mol. Cell. Biol.,* 10, 4529-37). Several investigators have demonstrated that nucleic acid molecules expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992, *Antisense Res. Dev.,* 2, 3-15; Ojwang et al., 1992, *Proc. Natl. Acad. Sci. USA,* 89, 10802-6; Chen et al., 1992, *Nucleic Acids Res.,* 20, 4581-9; Yu et al., 1993, *Proc. Natl. Acad. Sci. U S A,* 90, 6340-4; L'Huillier et al., 1992, *EMBO J.,* 11, 4411-8; Lisziewicz et al., 1993, *Proc. Natl. Acad. Sci. U. S. A,* 90, 8000-4; Thompson et al., 1995, *Nucleic Acids Res.,* 23, 2259; Sullenger & Cech, 1993, *Science,* 262, 1566). More specifically, transcription units such as the ones derived from genes encoding U6 small nuclear (snRNA), transfer RNA (tRNA) and adenovirus VA RNA are useful in generating high concentrations of desired RNA molecules such as siRNA in cells (Thompson et al., supra; Couture and Stinchcomb, 1996, supra; Noonberg et al., 1994, *Nucleic Acid Res.,* 22, 2830; Noonberg et al., U.S. Pat. No. 5,624,803; Good et al., 1997, *Gene Ther.,* 4, 45; Beigelman et al., International PCT Publication No. WO 96/18736. The above siRNA transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated virus vectors), or viral RNA vectors (such as retroviral or alphavirus vectors) (for a review see Couture and Stinchcomb, 1996, supra).

[0220] In another aspect the invention features an expression vector comprising a nucleic acid sequence encoding at least one of the siRNA molecules of the invention, in a manner that allows expression of that siRNA molecule. The expression vector comprises in one embodiment; a) a transcription initiation region; b) a transcription termination region; and c) a nucleic acid sequence encoding at least one strand of the siRNA molecule; wherein the sequence is operably linked to the initiation region and the termination region, in a manner that allows expression and/or delivery of the siRNA molecule.

[0221] In another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an open reading frame; and d) a nucleic acid sequence encoding at least one strand of a siRNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame; and wherein the sequence is operably linked to the initiation region, the open reading frame and the termination region, in a manner that allows expression and/or delivery of the siRNA molecule. In yet another embodiment the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; and d) a nucleic acid sequence encoding at least one siRNA molecule; wherein the sequence is operably linked to the initiation region, the intron and the termination region, in a manner which allows expression and/or delivery of the nucleic acid molecule.

[0222] In another embodiment, the expression vector comprises: a) a transcription initiation region; b) a transcription termination region; c) an intron; d) an open reading frame; and e) a nucleic acid sequence encoding at least one strand of a siRNA molecule, wherein the sequence is operably linked to the 3'-end of the open reading frame; and wherein the sequence is operably linked to the initiation region, the intron, the open reading frame and the termination region, in a manner which allows expression and/or delivery of the siRNA molecule.

EXAMPLES

[0223] The following are non-limiting examples showing the selection, isolation, synthesis and activity of nucleic acids of the instant invention.

Example 1

Tandem Synthesis of siRNA Constructs

[0224] Exemplary siRNA molecules of the invention are synthesized in tandem using a cleavable linker, for example a succinyl-based linker. Tandem synthesis as described herein is followed by a one step purification process that provides RNAi molecules in high yield. This approach is highly amenable to siRNA synthesis in support of high throughput RNAi screening, and can be readily adapted to multi-column or multi-well synthesis platforms.

[0225] After completing a tandem synthesis of an siRNA oligo and its compliment in which the 5'-terminal dimethoxytrityl (5'-O-DMT) group remains intact (trityl on synthesis), the oligonucleotides are deprotected as described above. Following deprotection, the siRNA sequence strands are allowed to spontaneously hybridize. This hybridization yields a duplex in which one strand has retained the 5'-O-DMT group while the complimentary strand comprises a terminal 5'-hydroxyl. The newly formed duplex to behaves as a single molecule during routine solid-phase extraction purification (Trityl-On purification) even though only one molecule has a dimethoxytrityl group. Because the strands form a stable duplex, this dimethoxytrityl group (or an equivalent group, such as other trityl groups or other hydrophobic moieties) is all that is required to purify the pair of oligos, for example by using a C18 cartridge.

[0226] Standard phosphoramidite synthesis chemistry is used up to point of introducing a tandem linker, such as an inverted deoxyabasic succinate linker (see **FIG. 1**) or an equivalent cleavable linker. A non-limiting example of linker coupling conditions that can be used includes a

hindered base such as diisopropylethylamine (DIPA) and/or DMAP in the presence of an activator reagent such as Bromotripyrrolidinophosphoniumhexaflurorophosphate (PyBrOP). After the linker is coupled, standard synthesis chemistry is utilized to complete synthesis of the second sequence leaving the terminal the 5'-O-DMT intact. Following synthesis, the resulting oligonucleotide is deprotected according to the procedures described herein and quenched with a suitable buffer, for example with 50 mM NaOAc or 1.5M $NH_4H_2CO_3$.

[0227] Purification of the siRNA duplex can be readily accomplished using solid phase extraction, for example using a Waters C18 SepPak 1 g cartridge conditioned with 1 column volume (CV) of acetonitrile, 2 CV H20, and 2 CV 50 mM NaOAc. The sample is loaded and then washed with 1 CV H20 or 50 mM NaOAc. Failure sequences are eluted with 1 CV 14% ACN (Aqueous with 50 mM NaOAc and 50 mM NaCl). The column is then washed, for example with 1 CV H20 followed by on-column detritylation, for example by passing 1 CV of 1% aqueous trifluoroacetic acid (TFA) over the column, then adding a second CV of 1% aqueous TFA to the column and allowing to stand for approx. 10 minutes. The remaining TFA solution is removed and the column washed with H20 followed by 1 CV 1M NaCl and additional H20. The siRNA duplex product is then eluted, for example using 1 CV 20% aqueous CAN.

[0228] **FIG. 2** provides an example of MALDI-TOV mass spectrometry analysis of a purified siRNA construct in which each peak corresponds to the calculated mass of an individual siRNA strand of the siRNA duplex. The same purified siRNA provides three peaks when analyzed by capillary gel electrophoresis (CGE), one peak presumably corresponding to the duplex siRNA, and two peaks presumably corresponding to the separate siRNA sequence strands. Ion exchange HPLC analysis of the same siRNA contract only shows a single peak.

Example 2

Identification of Potential siRNA Target Sites in any RNA Sequence

[0229] The sequence of an RNA target of interest, such as a HIV-1, is screened for target sites, for example by using a computer folding algorithm. In a non-limiting example, the sequence of gene or RNA gene transcripts derived from a database, such as Genbank Accession numbers shown in Table III, is used to generate siRNA targets having complimentarity to the target. Such sequences can be obtained from a database, or can be determined experimentally as known in the art. Target sites that are known, for example, those target sites determined to be effective target sites based on studies with other nucleic acid molecules, for example ribozymes or antisense, or those targets known to be associated with a disease or condition such as those sites containing mutations or deletions, can be used to design siRNA molecules targeting those sites as well. Various parameters can be used to determine which sites are the most suitable target sites within the target RNA sequence. These parameters include but are not limited to secondary or tertiary RNA structure, the nucleotide base composition of the target sequence, the degree of homology between various regions of the target sequence, or the relative position of the target sequence within the RNA transcript. Based on these determinations, any number of target sites within the RNA transcript can be chosen to screen siRNA molecules for efficacy, for example by using in vitro RNA cleavage assays, cell culture, or animal models. In a non-limiting example, anywhere from 1 to 1000 target sites are chosen within the transcript based on the size of the siRNA contruct to be used. High throughput screening assays can be developed for screening siRNA molecules using methods known in the art, such as with multi-well or multi-plate assays to determine efficient reduction in target gene expression.

Example 3

Selection of siRNA Molecule Target Sites in a RNA

[0230] The following non-limiting steps can be used to carry out the selection of siRNAs targeting a given gene sequence or transcript, eg HIV-1.

[0231] 1. The target sequence is parsed in silico into a list of all fragments or subsequences of a particular length, for example 23 nucleotide fragments, contained within the target sequence. This step is typically carried out using a custom Perl script, but commercial sequence analysis programs such as Oligo, MacVector, or the GCG Wisconsin Package can be employed as well.

[0232] 2. In some instances the siRNAs correspond to more than one target sequence; such would be the case for example in targeting many different strains of a viral sequence, for targeting different transcipts of the same gene, targeting different transcipts of more than one gene, or for targeting both the human gene and an animal homolog. In this case, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find matching sequences in each list. The subsequences are then ranked according to the number of target sequences that contain the given subsequence; the goal is to find subsequences that are present in most or all of the target sequences. Alternately, the ranking can indentify subsequences that are unique to a target sequence, such as a mutant target sequence. Such an approach would enable the use of siRNA to target specifically the mutant sequence and not effect the expression of the normal sequence.

[0233] 3. In some instances the siRNA subsequences are absent in one or more sequences while present in the desired target sequence; such would be the case if the siRNA targets a gene with a paralogous family member that is to remain untargeted. As in case 2 above, a subsequence list of a particular length is generated for each of the targets, and then the lists are compared to find sequences that are present in the target gene but are absent in the untargeted paralog.

[0234] 4. The ranked siRNA subsequences can be further analyzed and ranked according to GC content. A preference can be given to sites containing 30-70% GC, with a further preference to sites containing 40-60% GC.

[0235] 5. The ranked siRNA subsequences can be further analyzed and ranked according to self-folding and internal hairpins. Weaker internal folds are preferred; strong hairpin structures are to be avoided.

[0236] 6. The ranked siRNA subsequences can be further analyzed and ranked according to whether they have runs of GGG or CCC in the sequence. GGG (or even more Gs) in either strand can make oligonucleotide synthesis problematic, so it is avoided whenever better sequences are available. CCC is searched in the target strand because that will place GGG in the antisense strand.

[0237] 7. The ranked siRNA subsequences can be further analyzed and ranked according to whether they have the dinucleotide UU (uridine dinucleotide) on the 3' end of the sequence, and/or AA on the 5' end of the sequence (to yield 3' UU on the antisense sequence). These sequences allow one to design siRNA molecules with terminal TT thymidine dinucleotides.

[0238] 8. Four or five target sites are chosen from the ranked list of subsequences as described above. For example, in subsequences having 23 nucleotides, the right 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the upper (sense) strand of the siRNA duplex, while the reverse complement of the left 21 nucleotides of each chosen 23-mer subsequence are then designed and synthesized for the lower (antisense) strand of the siRNA duplex. If terminal TT residues are desired for the sequence (as described in paragraph 7), then the two 3' terminal nucleotides of both the sense and antisense strands are replaced by TT prior to synthesizing the oligos.

[0239] 9. The siRNA molecules are screened in an in vitro, cell culture or animal model system to identify the most active siRNA molecule or the most preferred target site within the target RNA sequence.

[0240] In an alternate approach, a pool of siRNA constructs specific to a HIV target sequence is used to screen for target sites in cells expressing HIV RNA. The general strategy used in this approach is shown in **FIG. 9**. A non-limiting example of such as pool is a pool comprising sequences having sense sequences comprising SEQ ID NOs. 1-738 and antisense sequences comprising SEQ ID NOs. 739-1476 respectively. Cells expressing HIV are transfected with the pool of siRNA constructs and cells that demonstrate a phenotype associated with HIV inhibition are sorted. The pool of siRNA constructs can be expressed from transcription cassettes inserted into appropriate vectors (see for example **FIG. 7** and **FIG. 8**). Cells in which HIV expression is decreased due to siRNA treatment demonstrate a phenotypic change, for example decreased production of HIV RNA or HIV protein(s) compared to untreated cells or cells treated with a control siRNA. The siRNA from cells demonstrating a positive phenotypic change (e.g., decreased HIV RNA or protein), are sequenced to determine the most suitable target site(s) within the target HIV RNA sequence.

Example 4

HIV Targeted siRNA Design

[0241] siRNA target sites were chosen by analyzing sequences of the HIV-1 RNA target (for example Genbank Accession Nos. shown in Table III) and optionally prioritizing the target sites on the basis of folding (structure of any given sequence analyzed to determine siRNA accessibility to the target). The sequence alignments of all known A and B strains of HIV were screened for homology and siRNA molecules were designed to target conserved sequences across these strains since the A and B strains are currently the most prevalent strains. Alternately, all known strains or other subclasses of HIV can be similarly screened for homology (see Table IV) and homologous sequences used as targets. A cutoff for % homology between the different strains can be used to increase or decrease the number of targets considered, for example 70%, 75%, 80%, 85%, 90% or 95% homology. The sequences shown in Table I represent 80% homology between the HIV strains shown in Table III. siRNA molecules were designed that could bind each target sequence and are optionally individually analyzed by computer folding to assess whether the siRNA molecule can interact with the target sequence. Varying the length of the siRNA molecules can be chosen to optimize activity. The siRNA sense (upper sequence) and antisense (lower sequence) sequences shown in Table I comprise 19 nucleotides in length, with the sense strand comprising the same sequence as the target sequence and the antisense strand comprising a complimentary sequence to the sense/target sequence. The sense and antisense strands can further comprise nucleotide 3'-overhangs as described herein, preferably the overhangs comprise about 2 nucleotides which can optionally be complimentary to the target sequence in the antisense siRNA strand, and/or optionally analogous to the adjacent nucleotides in the target sequence when present in the sense siRNA strand. Generally, a sufficient number of complimentary nucleotide bases are chosen to bind to, or otherwise interact with, the target RNA, but the degree of complementarity can be modulated to accommodate siRNA duplexes or varying length or base composition. By using such methodologies, siRNA molecules can be designed to target sites within any known RNA sequence, for example those RNA sequences corresponding to the any gene transcript.

Example 5

Chemical Synthesis and Purification of siRNA

[0242] siRNA molecules can be designed to interact with various sites in the RNA message, for example target sequences within the RNA sequences described herein. The sequence of one strand of the siRNA molecule(s) are complementary to the target site sequences described above. The siRNA molecules can be chemically synthesized using methods described herein. Inactive siRNA molecules that are used as control sequences can be synthesized by scrambling the sequence of the siRNA molecules such that it is not complimentary to the target sequence.

Example 6

RNAi in vitro Assay to Assess siRNA Activity

[0243] An in vitro assay that recapitulates RNAi in a cell free system is used to evaluate siRNA constructs targeting HIV RNA targets. The assay comprises the system described by Tuschl et al., 1999, *Genes and Development,* 13, 3191-3197 and Zamore et al., 2000, *Cell,* 101, 25-33 adapted for use with HIV target RNA. A Drosophila extract derived from syncytial blastoderm is used to reconstitute RNAi activity in

vitro. Target RNA is generated via in vitro transcription from an appropriate HIV expressing plasmid using T7 RNA polymerase. The target RNA can also be synthesized chemically as described herein. Sense and antisense siRNA strands (for example 20 uM each) are annealed by incubation in buffer (such as 100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 1 min. at 90° C. followed by 1 hour at 37° C., then diluted in lysis buffer (for example 100 mM potassium acetate, 30 mM HEPES-KOH at pH 7.4, 2 mM magnesium acetate). Annealing can be monitored by gel electrophoresis on an agarose gel in TBE buffer and stained with ethidium bromide. The Drosophila lysate is prepared using zero to two hour old embryos from Oregon R flies collected on yeasted molasses agar that are dechorionated and lysed. The lysate is centrifuged and the supernatant isolated. The assay comprises a reaction mixture containing 50% lysate [vol/vol], RNA (10-50 pM final concentration), and 10% [vol/vol] lysis buffer containing siRNA (10 nM final concentration). The reaction mixture also contains 10 mM creatine phosphate, 10 ug.ml creatine phosphokinase, 100 um GTP, 100 uM UTP, 100 uM CTP, 500 uM ATP, 5 mM DTT, 0.1 U/uL RNasin (Promega), and 100 uM of each amino acid. The final concentration of potassium acetate is adjusted to 100 mM. The reactions are pre-assembled on ice and preincubated at 25° C. for 10 minutes before adding RNA, then incubated at 25° C. for an additional 60 minutes. Reactions are quenched with 4 volumes of 1.25×Passive Lysis Buffer (Promega). Target RNA cleavage is assayed by RT-PCR analysis or other methods known in the art and are compared to control reactions in which siRNA is omitted from the reaction.

[0244] Alternately, internally-labeled target RNA for the assay is prepared by in vitro transcription in the presence of [a-^{32}P] CTP, passed over a G 50 Sephadex column by spin chromatography and used as target RNA without further purification. Optionally, target RNA is 5'-^{32}P-end labeled using T4 polynucleotide kinase enzyme. Assays are performed as described above and target RNA and the specific RNA cleavage products generated by RNAi are visualized on an autoradiograph of a gel. The percentage of cleavage is determined by Phosphor Imager® quantitation of bands representing intact control RNA or RNA from control reactions without siRNA and the cleavage products generated by the assay.

Example 7

Cell Culture

[0245] The siRNA constructs of the invention can be used in various cell culture systems as are commonly known in the art to screen for compounds having anti-HIV activity. B cell, T cell, macrophage and endothelial cell culture systems are non-limiting examples of cell culture systems that can be readily adapted for screening siRNA molecules of the invention. In a non-limiting example, siRNA molecules of the invention are co-transfected with HIV-1 pNL4-3 proviral DNA into 293/EcR cells as described by Lee et al., 2002, *Nature Biotechnology,* 19, 500-505, using a U6 snRNA promoter driven expression system.

[0246] In a non-limiting example, the siRNA expression vectors are prepared using the pTZ U6+1 vector described in Lee et al. supra. as follows. One cassette harbors the 21-nucleotide sense sequences and the other a 21-nucleotide antisense sequence (Table I). These sequences are designed to target HIV-1 RNA targets described herein. As a control to verify a siRNA mechanism, irrelevant sense and antisense (S/AS) sequences lacking complementarity to HIV-1 (S/AS (IR)) are subcloned in pTZ U6+1. RNA samples are prepared from 293/EcR cells transiently co-transfected with siRNA or control constructs, and subjected to Ponasterone A induction. RNAs are also prepared from 293 cells co-transfected with HIV-1 pNL4-3 proviral DNA and siRNA or control constructs. For determination of anti-HIV-1 activity of the siRNAs, transient assays are done by co-transfection of siRNA constructs and infectious HIV-1 proviral DNA, pNL4-3 into 293 cells as described above, followed by Northern analysis as known in the art. The p24 values are calculated with the aid of, for example, a Dynatech MR5000 ELISA plate reader (Dynatech Labs Inc., Chantilly, Va.). Cell viability can also be assessed using a Trypan Blue dye exclusion count at four days after transfection.

[0247] Other cell culture model systems are generally known in the art, see for example Duzgunes et al., 2001, Nucleosides, *Nucleotides & Nucleic Acids,* 20(4-7), 515-523; Cagnun et al., 2000, *Antisense Nucleic Acid Drug Dev.,* 10, 251; Ho et al., 1995, *Stem Cells,* 13 supp 3, 100; and Baur et al., 1997, *Blood,* 89, 2259. These cell culture systems can be readily adapted for use with the compositions of the instant invention.

[0248] Animal Models

[0249] The siRNA constructs of the invention can be evaluated in a variety of animal models, including for example a hollow fiber HIV model (see for example Gruenberg, U.S. Pat. No. 5,627,070), mouse models for AIDS using transgenic mice expressing HIV-1 genes from CD4 promoters and enhancers (see for example Jolicoeur, International PCT Publication No. WO 98/50535) and/or the HIV/SIV/SHIV non-human primate models (see for example Narayan, U.S. Pat. No. 5,849,994). The siRNA compounds and virus can be administered by a variety of methods and routes as described herein and as known in the art. Quantitation of results in these models can be performed by a variety of methods, including quantitative PCR, quantitative and bulk co-cultivation assays, plasma co-cultivation assays, antigen and antibody detection assays, lymphocyte proliferation, intracellular cytokines, flow cytometry, as well as hematology and CBC evaluation. Additional animal models are generally known in the art, see for example Bai et al., 2000, *Mol. Ther.,* 1, 244.

[0250] Indications

[0251] Particular degenerative and disease states that can be associated with HIV expression modulation include but are not limited to acquired immunodeficiency disease (AIDS) and related diseases and conditions, including but not limited to Kaposi's sarcoma, lymphoma, cervical cancer, squamous cell carcinoma, cardiac myopathy, rheumatic diseases, and opportunistic infection, for example *Pneumocystis carinii,* Cytomegalovirus, Herpes simplex, Mycobacteria, Cryptococcus, Toxoplasma, Progressive multifocal leucoencephalopathy (Papovavirus), Mycobacteria, Aspergillus, Cryptococcus, Candida, Cryptosporidium, *Isospora belli,* Microsporidia and any other diseases or conditions that are related to or will respond to the levels of HIV in a cell or tissue, alone or in combination with other therapies

[0252] The present body of knowledge in HIV research indicates the need for methods to assay HIV activity and for compounds that can regulate HIV expression for research, diagnostic, and therapeutic use.

[0253] The use of antiviral compounds, monoclonal antibodies, chemotherapy, radiation therapy, analgesics, and/or anti-inflammatory compounds, are all non-limiting examples of a methods that can be combined with or used in conjunction with the nucleic acid molecules (e.g. ribozymes and antisense molecules) of the instant invention. Examples of antiviral compounds that can be used in conjunction with the nucleic acid molecules of the invention include but are not limited to AZT (also known as zidovudine or ZDV), ddC (zalcitabine), ddI (dideoxyinosine), d4T (stavudine), and 3TC (lamivudine) Ribavirin, delvaridine (Rescriptor), nevirapine (Viramune), efravirenz (Sustiva), ritonavir (Norvir), saquinivir (Invirase), indinavir (Crixivan), amprenivir (Agenerase), nelfinavir (Viracept), and/or lopinavir (Kaletra). Common chemotherapies that can be combined with nucleic acid molecules of the instant invention include various combinations of cytotoxic drugs to kill cancer cells. These drugs include but are not limited to paclitaxel (Taxol), docetaxel, cisplatin, methotrexate, cyclophosphamide, doxorubin, fluorouracil carboplatin, edatrexate, gemcitabine, vinorelbine etc. Those skilled in the art will recognize that other drug compounds and therapies can be similarly be readily combined with the nucleic acid molecules of the instant invention (e.g. ribozymes, siRNA and antisense molecules) are hence within the scope of the instant invention.

[0254] Diagnostic Uses

[0255] The siRNA molecules of the invention can be used in a variety of diagnostic applications, such as in identifying molecular targets such as RNA in a variety of applications, for example, in clinical, industrial, environmental, agricultural and/or research settings. Such diagnostic use of siRNA molecules involves utilizing reconstituted RNAi systems, for example using cellular lysates or partially purified cellular lysates. siRNA molecules of this invention can be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of endogenous or exogenous, for example viral, RNA in a cell. The close relationship between siRNA activity and the structure of the target RNA allows the detection of mutations in any region of the molecule, which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple siRNA molecules described in this invention, one can map nucleotide changes, which are important to RNA structure and function in vitro, as well as in cells and tissues. Cleavage of target RNAs with siRNA molecules can be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease or infection. In this manner, other genetic targets can be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combination therapies (e.g., multiple siRNA molecules targeted to different genes, siRNA molecules coupled with known small molecule inhibitors, or intermittent treatment with combinations siRNA molecules and/or other chemical or biological molecules). Other in vitro uses of siRNA molecules of this invention are well known in the art, and include detection of the presence of mRNAs associated with a disease, infection, or related condition. Such RNA is detected by determining the presence of a cleavage product after treatment with a siRNA using standard methodologies, for example fluorescence resonance emission transfer (FRET).

[0256] In a specific example, siRNA molecules that can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first siRNA molecules is used to identify wild-type RNA present in the sample and the second siRNA molecules will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both siRNA molecules to demonstrate the relative siRNA efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two siRNA molecules, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (i.e., disease related or infection related) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

[0257] All patents and publications mentioned in the specification are indicative of the levels of skill of those skilled in the art to which the invention pertains. All references cited in this disclosure are incorporated by reference to the same extent as if each reference had been incorporated by reference in its entirety individually.

[0258] One skilled in the art would readily appreciate that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those inherent therein. The methods and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the spirit of the invention, are defined by the scope of the claims.

[0259] It will be readily apparent to one skilled in the art that varying substitutions and modifications can be made to the invention disclosed herein without departing from the scope and spirit of the invention. Thus, such additional embodiments are within the scope of the present invention and the following claims.

[0260] The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms. The terms and expressions which have been

employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

[0261] In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

TABLE I

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
UUUGGAAAGGACCAGCAAA	1	UUUGGAAAGGACCAGCAAA	1	UUUGCUGGUCCUUUCCAAA	739
CAGGAGCAGAUGAUACAGU	2	CAGGAGCAGAUGAUACAGU	2	ACUGUAUCAUCUGCUCCUG	740
AGAAAAGGGGGGAUUGGGG	3	AGAAAAGGGGGGAUUGGGG	3	CCCCAAUCCCCCCUUUUCU	741
GUAGACAGGAUGAGGAUUA	4	GUAGACAGGAUGAGGAUUA	4	UAAUCCUCAUCCUGUCUAC	742
ACAGGAGCAGAUGAUACAG	5	ACAGGAGCAGAUGAUACAG	5	CUGUAUCAUCUGCUCCUGU	743
GAAAAGGGGGGAUUGGGGG	6	GAAAAGGGGGGAUUGGGGG	6	CCCCCAAUCCCCCCUUUUC	744
UUAGAUACAGGAGCAGAUG	7	UUAGAUACAGGAGCAGAUG	7	CAUCUGCUCCUGUAUCUAA	745
UAGAUACAGGAGCAGAUGA	8	UAGAUACAGGAGCAGAUGA	8	UCAUCUGCUCCUGUAUCUA	746
AGCAGAAGACAGUGGCAAU	9	AGCAGAAGACAGUGGCAAU	9	AUUGCCACUGUCUUCUGCU	747
AUUAGAUACAGGAGCAGAU	10	AUUAGAUACAGGAGCAGAU	10	AUCUGCUCCUGUAUCUAAU	748
AUACAGGAGCAGAUGAUAC	11	AUACAGGAGCAGAUGAUAC	11	GUAUCAUCUGCUCCUGUAU	749
GAGCAGAAGACAGUGGCAA	12	GAGCAGAAGACAGUGGCAA	12	UUGCCACUGUCUUCUGCUC	750
AGAGCAGAAGACAGUGGCA	13	AGAGCAGAAGACAGUGGCA	13	UGCCACUGUCUUCUGCUCU	751
GCAGAAGACAGUGGCAAUG	14	GCAGAAGACAGUGGCAAUG	14	CAUUGCCACUGUCUUCUGC	752
AGAUACAGGAGCAGAUGAU	15	AGAUACAGGAGCAGAUGAU	15	AUCAUCUGCUCCUGUAUCU	753
UACAGGAGCAGAUGAUACA	16	UACAGGAGCAGAUGAUACA	16	UGUAUCAUCUGCUCCUGUA	754
UAUUAGAUACAGGAGCAGA	17	UAUUAGAUACAGGAGCAGA	17	UCUGCUCCUGUAUCUAAUA	755
GAUACAGGAGCAGAUGAUA	18	GAUACAGGAGCAGAUGAUA	18	UAUCAUCUGCUCCUGUAUC	756
AUGGAAAACAGAUGGCAGG	19	AUGGAAAACAGAUGGCAGG	19	CCUGCCAUCUGUUUUCCAU	757
GUCAACAUAAUUGGAAGAA	20	GUCAACAUAAUUGGAAGAA	20	UUCUUCCAAUUAUGUUGAC	758
UAUGGAAAACAGAUGGCAG	21	UAUGGAAAACAGAUGGCAG	21	CUGCCAUCUGUUUUCCAUA	759
AUGAUAGGGGGAAUUGGAG	22	AUGAUAGGGGGAAUUGGAG	22	CUCCAAUUCCCCCUAUCAU	760
CAGAAGACAGUGGCAAUGA	23	CAGAAGACAGUGGCAAUGA	23	UCAUUGCCACUGUCUUCUG	761
CAAUGGCCAUUGACAGAAG	24	CAAUGGCCAUUGACAGAAG	24	CUUCUGUCAAUGGCCAUUG	762
UCAACAUAAUUGGAAGAAA	25	UCAACAUAAUUGGAAGAAA	25	UUUCUUCCAAUUAUGUUGA	763
AAUGGCCAUUGACAGAAGA	26	AAUGGCCAUUGACAGAAGA	26	UCUUCUGUCAAUGGCCAUU	764
UGAUAGGGGGAAUUGGAGG	27	UGAUAGGGGGAAUUGGAGG	27	CCUCCAAUUCCCCCUAUCA	765
GACAGGCUAAUUUUUUAGG	28	GACAGGCUAAUUUUUUAGG	28	CCUAAAAAAUUAGCCUGUC	766
AUUUUCGGGUUUAUUACAG	29	AUUUUCGGGUUUAUUACAG	29	CUGUAAUAAACCCGAAAAU	767

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
CUAUUAGAUACAGGAGCAG	30	CUAUUAGAUACAGGAGCAG	30	CUGCUCCUGUAUCUAAUAG	768
AGACAGGCUAAUUUUUUAG	31	AGACAGGCUAAUUUUUUAG	31	CUAAAAAAUUAGCCUGUCU	769
AAAUGAUAGGGGGAAUUGG	32	AAAUGAUAGGGGGAAUUGG	32	CCAAUUCCCCCUAUCAUUU	770
UAUGGGCAAGCAGGGAGCU	33	UAUGGGCAAGCAGGGAGCU	33	AGCUCCCUGCUUGCCCAUA	771
UAGUAUGGGCAAGCAGGGA	34	UAGUAUGGGCAAGCAGGGA	34	UCCCUGCUUGCCCAUACUA	772
GAAAACAGAUGGCAGGUGA	35	GAAAACAGAUGGCAGGUGA	35	UCACCUGCCAUCUGUUUUC	773
ACCAUCAAUGAGGAAGCUG	36	ACCAUCAAUGAGGAAGCUG	36	CAGCUUCCUCAUUGAUGGU	774
AAUGAUAGGGGGAAUUGGA	37	AAUGAUAGGGGGAAUUGGA	37	UCCAAUUCCCCCUAUCAUU	775
UGGAAAACAGAUGGCAGGU	38	UGGAAAACAGAUGGCAGGU	38	ACCUGCCAUCUGUUUUCCA	776
GGAAAACAGAUGGCAGGUG	39	GGAAAACAGAUGGCAGGUG	39	CACCUGCCAUCUGUUUUCC	777
GAUUAUGGAAAACAGAUGG	40	GAUUAUGGAAAACAGAUGG	40	CCAUCUGUUUUCCAUAAUC	778
AAAAUGAUAGGGGGAAUUG	41	AAAAUGAUAGGGGGAAUUG	41	CAAUUCCCCCUAUCAUUUU	779
UGGAAAGGUGAAGGGGCAG	42	UGGAAAGGUGAAGGGGCAG	42	CUGCCCCUUCACCUUUCCA	780
AUCAAUGAGGAAGCUGCAG	43	AUCAAUGAGGAAGCUGCAG	43	CUGCAGCUUCCUCAUUGAU	781
UGGAAACCAAAAAUGAUAG	44	UGGAAACCAAAAAUGAUAG	44	CUAUCAUUUUUGGUUUCCA	782
CCAUCAAUGAGGAAGCUGC	45	CCAUCAAUGAGGAAGCUGC	45	GCAGCUUCCUCAUUGAUGG	783
AGGGAUUAUGGAAAACAGA	46	AGGGAUUAUGGAAAACAGA	46	UCUGUUUUCCAUAAUCCCU	784
GGAAACCAAAAAUGAUAGG	47	GGAAACCAAAAAUGAUAGG	47	CCUAUCAUUUUUGGUUUCC	785
UAGGGGGAAUUGGAGGUUU	48	UAGGGGGAAUUGGAGGUUU	48	AAACCUCCAAUUCCCCCUA	786
UACAGUGCAGGGGAAAGAA	49	UACAGUGCAGGGGAAAGAA	49	UUCUUUCCCCUGCACUGUA	787
CUCUAUUAGAUACAGGAGC	50	CUCUAUUAGAUACAGGAGC	50	GCUCCUGUAUCUAAUAGAG	788
GGAUUAUGGAAAACAGAUG	51	GGAUUAUGGAAAACAGAUG	51	CAUCUGUUUUCCAUAAUCC	789
CCAAAAAUGAUAGGGGGAA	52	CCAAAAAUGAUAGGGGGAA	52	UUCCCCCUAUCAUUUUUGG	790
AUGGAAACCAAAAAUGAUA	53	AUGGAAACCAAAAAUGAUA	53	UAUCAUUUUUGGUUUCCAU	791
CAGUGCAGGGGAAAGAAUA	54	CAGUGCAGGGGAAAGAAUA	54	UAUUCUUUCCCCUGCACUG	792
ACAAUGGCCAUUGACAGAA	55	ACAAUGGCCAUUGACAGAA	55	UUCUGUCAAUGGCCAUUGU	793
CCAUGCAUGGACAAGUAGA	56	CCAUGCAUGGACAAGUAGA	56	UCUACUUGUCCAUGCAUGG	794
AUUAUGGAAAACAGAUGGC	57	AUUAUGGAAAACAGAUGGC	57	GCCAUCUGUUUUCCAUAAU	795
AACAAUGGCCAUUGACAGA	58	AACAAUGGCCAUUGACAGA	58	UCUGUCAAUGGCCAUUGUU	796
AAAAAUGAUAGGGGGAAUU	59	AAAAAUGAUAGGGGGAAUU	59	AAUUCCCCCUAUCAUUUUU	797
GCCAUGCAUGGACAAGUAG	60	GCCAUGCAUGGACAAGUAG	60	CUACUUGUCCAUGCAUGGC	798
UAGCAGGAAGAUGGCCAGU	61	UAGCAGGAAGAUGGCCAGU	61	ACUGGCCAUCUUCCUGCUA	799
CAAAAAUGAUAGGGGGAAU	62	CAAAAAUGAUAGGGGGAAU	62	AUUCCCCCUAUCAUUUUUG	800
AAGAAAUGAUGACAGCAUG	63	AAGAAAUGAUGACAGCAUG	63	CAUGCUGUCAUCAUUUCUU	801
UCUAUUAGAUACAGGAGCA	64	UCUAUUAGAUACAGGAGCA	64	UGCUCCUGUAUCUAAUAGA	802

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GCUCUAUUAGAUACAGGAG	65	GCUCUAUUAGAUACAGGAG	65	CUCCUGUAUCUAAUAGAGC	803
CAGGCUAAUUUUUUAGGGA	66	CAGGCUAAUUUUUUAGGGA	66	UCCCUAAAAAAUUAGCCUG	804
AGGAGCAGAUGAUACAGUA	67	AGGAGCAGAUGAUACAGUA	67	UACUGUAUCAUCUGCUCCU	805
AAACAAUGGCCAUUGACAG	68	AAACAAUGGCCAUUGACAG	68	CUGUCAAUGGCCAUUGUUU	806
CGGGUUUAUUACAGGGACA	69	CGGGUUUAUUACAGGGACA	69	UGUCCCUGUAAUAAACCCG	807
CAACAUAAUUGGAAGAAAU	70	CAACAUAAUUGGAAGAAAU	70	AUUUCUUCCAAUUAUGUUG	808
UCAAUGAGGAAGCUGCAGA	71	UCAAUGAGGAAGCUGCAGA	71	UCUGCAGCUUCCUCAUUGA	809
GGAAAGGUGAAGGGGCAGU	72	GGAAAGGUGAAGGGGCAGU	72	ACUGCCCCUUCACCUUUCC	810
UUUCGGGUUUAUUACAGGG	73	UUUCGGGUUUAUUACAGGG	73	CCCUGUAAUAAACCCGAAA	811
UCGGGUUUAUUACAGGGAC	74	UCGGGUUUAUUACAGGGAC	74	GUCCCUGUAAUAAACCCGA	812
ACAGUGCAGGGGAAAGAAU	75	ACAGUGCAGGGGAAAGAAU	75	AUUCUUUCCCCUGCACUGU	813
AUGCAUGGACAAGUAGACU	76	AUGCAUGGACAAGUAGACU	76	AGUCUACUUGUCCAUGCAU	814
AAGCCAUGCAUGGACAAGU	77	AAGCCAUGCAUGGACAAGU	77	ACUUGUCCAUGCAUGGCUU	815
AGCCAUGCAUGGACAAGUA	78	AGCCAUGCAUGGACAAGUA	78	UACUUGUCCAUGCAUGGCU	816
GCAUUAUCAGAAGGAGCCA	79	GCAUUAUCAGAAGGAGCCA	79	UGGCUCCUUCUGAUAAUGC	817
AAUUGGAGAAGUGAAUUAU	80	AAUUGGAGAAGUGAAUUAU	80	AUAAUUCACUUCUCCAAUU	818
AGAAAAAAUCAGUAACAGU	81	AGAAAAAAUCAGUAACAGU	81	ACUGUUACUGAUUUUUUCU	819
GAAGCCAUGCAUGGACAAG	82	GAAGCCAUGCAUGGACAAG	82	CUUGUCCAUGCAUGGCUUC	820
ACAGGCUAAUUUUUUAGGG	83	ACAGGCUAAUUUUUUAGGG	83	CCCUAAAAAAUUAGCCUGU	821
GAAGAAAUGAUGACAGCAU	84	GAAGAAAUGAUGACAGCAU	84	AUGCUGUCAUCAUUUCUUC	822
UUUUCGGGUUUAUUACAGG	85	UUUUCGGGUUUAUUACAGG	85	CCUGUAAUAAACCCGAAAA	823
ACCAAAAAAUGAUAGGGGGA	86	ACCAAAAAAUGAUAGGGGGA	86	UCCCCCUAUCAUUUUUGGU	824
GAAGUGACAUAGCAGGAAC	87	GAAGUGACAUAGCAGGAAC	87	GUUCCUGCUAUGUCACUUC	825
UUCGGGUUUAUUACAGGGA	88	UUCGGGUUUAUUACAGGGA	88	UCCCUGUAAUAAACCCGAA	826
AUAGGGGGAAUUGGAGGUU	89	AUAGGGGGAAUUGGAGGUU	89	AACCUCCAAUUCCCCCUAU	827
AGAAGAAAUGAUGACAGCA	90	AGAAGAAAUGAUGACAGCA	90	UGCUGUCAUCAUUUCUUCU	828
AUUGGAGAAGUGAAUUAUA	91	AUUGGAGAAGUGAAUUAUA	91	UAUAAUUCACUUCUCCAAU	829
GGAAGUGACAUAGCAGGAA	92	GGAAGUGACAUAGCAGGAA	92	UUCCUGCUAUGUCACUUCC	830
AGGCUAAUUUUUUAGGGAA	93	AGGCUAAUUUUUUAGGGAA	93	UUCCCUAAAAAAUUAGCCU	831
UUAUGGAAAACAGAUGGCA	94	UUAUGGAAAACAGAUGGCA	94	UGCCAUCUGUUUUCCAUAA	832
GGGAUUAUGGAAAACAGAU	95	GGGAUUAUGGAAAACAGAU	95	AUCUGUUUUCCAUAAUCCC	833
UAGAAGAAAUGAUGACAGC	96	UAGAAGAAAUGAUGACAGC	96	GCUGUCAUCAUUUCUUCUA	834
AGCUCUAUUAGAUACAGGA	97	AGCUCUAUUAGAUACAGGA	97	UCCUGUAUCUAAUAGAGCU	835
GUAUGGGCAAGCAGGGAGC	98	GUAUGGGCAAGCAGGGAGC	98	GCUCCCUGCUUGCCCAUAC	836
CUUAGGCAUCUCCUAUGGC	99	CUUAGGCAUCUCCUAUGGC	99	GCCAUAGGAGAUGCCUAAG	837
GCAGGAACUACUAGUACCC	100	GCAGGAACUACUAGUACCC	100	GGGUACUAGUAGUUCCUGC	838

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GGGGAAGUGACAUAGCAGG	101	GGGGAAGUGACAUAGCAGG	101	CCUGCUAUGUCACUUCCCC	839
UACAAUCCCCAAAGUCAAG	102	UACAAUCCCCAAAGUCAAG	102	CUUGACUUUGGGGAUUGUA	840
UUCCCUACAAUCCCCAAAG	103	UUCCCUACAAUCCCCAAAG	103	CUUUGGGGAUUGUAGGGAA	841
AAGCUCUAUUAGAUACAGG	104	AAGCUCUAUUAGAUACAGG	104	CCUGUAUCUAAUAGAGCUU	842
CCUAUGGCAGGAAGAAGCG	105	CCUAUGGCAGGAAGAAGCG	105	CGCUUCUUCCUGCCAUAGG	843
AGGGGAAGUGACAUAGCAG	106	AGGGGAAGUGACAUAGCAG	106	CUGCUAUGUCACUUCCCCU	844
UCCUAUGGCAGGAAGAAGC	107	UCCUAUGGCAGGAAGAAGC	107	GCUUCUUCCUGCCAUAGGA	845
CAGCAUUAUCAGAAGGAGC	108	CAGCAUUAUCAGAAGGAGC	108	GCUCCUUCUGAUAAUGCUG	846
AUCUCCUAUGGCAGGAAGA	109	AUCUCCUAUGGCAGGAAGA	109	UCUUCCUGCCAUAGGAGAU	847
AGCAGGAACUACUAGUACC	110	AGCAGGAACUACUAGUACC	110	GGUACUAGUAGUUCCUGCU	848
GAAACCAAAAAUGAUAGGG	111	GAAACCAAAAAUGAUAGGG	111	CCCUAUCAUUUUUGGUUUC	849
AAACCAAAAAUGAUAGGGG	112	AAACCAAAAAUGAUAGGGG	112	CCCCUAUCAUUUUUGGUUU	850
CAGAAGGAGCCACCCCACA	113	CAGAAGGAGCCACCCCACA	113	UGUGGGGUGGCUCCUUCUG	851
UAGCAGGAACUACUAGUAC	114	UAGCAGGAACUACUAGUAC	114	GUACUAGUAGUUCCUGCUA	852
UGCAUGGACAAGUAGACUG	115	UGCAUGGACAAGUAGACUG	115	CAGUCUACUUGUCCAUGCA	853
UUAGGCAUCUCCUAUGGCA	116	UUAGGCAUCUCCUAUGGCA	116	UGCCAUAGGAGAUGCCUAA	854
UAUGGCAGGAAGAAGCGGA	117	UAUGGCAGGAAGAAGCGGA	117	UCCGCUUCUUCCUGCCAUA	855
AUAGCAGGAACUACUAGUA	118	AUAGCAGGAACUACUAGUA	118	UACUAGUAGUUCCUGCUAU	856
UAGACAUAAUAGCAACAGA	119	UAGACAUAAUAGCAACAGA	119	UCUGUUGCUAUUAUGUCUA	857
CAUUAUCAGAAGGAGCCAC	120	CAUUAUCAGAAGGAGCCAC	120	GUGGCUCCUUCUGAUAAUG	858
CUAUGGCAGGAAGAAGCGG	121	CUAUGGCAGGAAGAAGCGG	121	CCGCUUCUUCCUGCCAUAG	859
GAUAGGGGGAAUUGGAGGU	122	GAUAGGGGGAAUUGGAGGU	122	ACCUCCAAUUCCCCCUAUC	860
ACAAUCCCCAAAGUCAAGG	123	ACAAUCCCCAAAGUCAAGG	123	CCUUGACUUUGGGGAUUGU	861
AUUCCCUACAAUCCCCAAA	124	AUUCCCUACAAUCCCCAAA	124	UUUGGGGAUUGUAGGGAAU	862
AACCAAAAAUGAUAGGGGG	125	AACCAAAAAUGAUAGGGGG	125	CCCCCUAUCAUUUUUGGUU	863
UCUCCUAUGGCAGGAAGAA	126	UCUCCUAUGGCAGGAAGAA	126	UUCUUCCUGCCAUAGGAGA	864
CAUGCAUGGACAAGUAGAC	127	CAUGCAUGGACAAGUAGAC	127	GUCUACUUGUCCAUGCAUG	865
CCUGUGUACCCACAGACCC	128	CCUGUGUACCCACAGACCC	128	GGGUCUGUGGGUACACAGG	866
CAUCAAUGAGGAAGCUGCA	129	CAUCAAUGAGGAAGCUGCA	129	UGCAGCUUCCUCAUUGAUG	867
GACAUAGCAGGAACUACUA	130	GACAUAGCAGGAACUACUA	130	UAGUAGUUCCUGCUAUGUC	868
GAAAGGUGAAGGGGCAGUA	131	GAAAGGUGAAGGGGCAGUA	131	UACUGCCCCUUCACCUUUC	869
AGUGACAUAGCAGGAACUA	132	AGUGACAUAGCAGGAACUA	132	UAGUUCCUGCUAUGUCACU	870
GCAGAUGAUACAGUAUUAG	133	GCAGAUGAUACAGUAUUAG	133	CUAAUACUGUAUCAUCUGC	871
GGAGCAGAUGAUACAGUAU	134	GGAGCAGAUGAUACAGUAU	134	AUACUGUAUCAUCUGCUCC	872
CCAAGGGGAAGUGACAUAG	135	CCAAGGGGAAGUGACAUAG	135	CUAUGUCACUUCCCCUUGG	873

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GAAGCUCUAUUAGAUACAG	136	GAAGCUCUAUUAGAUACAG	136	CUGUAUCUAAUAGAGCUUC	874
GGGAAGUGACAUAGCAGGA	137	GGGAAGUGACAUAGCAGGA	137	UCCUGCUAUGUCACUUCCC	875
CAUGCCUGUGUACCCACAG	138	CAUGCCUGUGUACCCACAG	138	CUGUGGGUACACAGGCAUG	876
GAAAGAGCAGAAGACAGUG	139	GAAAGAGCAGAAGACAGUG	139	CACUGUCUUCUGCUCUUUC	877
ACAUAGCAGGAACUACUAG	140	ACAUAGCAGGAACUACUAG	140	CUAGUAGUUCCUGCUAUGU	878
CAUCUCCUAUGGCAGGAAG	141	CAUCUCCUAUGGCAGGAAG	141	CUUCCUGCCAUAGGAGAUG	879
GAGCAGAUGAUACAGUAUU	142	GAGCAGAUGAUACAGUAUU	142	AAUACUGUAUCAUCUGCUC	880
AGCAUUAUCAGAAGGAGCC	143	AGCAUUAUCAGAAGGAGCC	143	GGCUCCUUCUGAUAAUGCU	881
CACCAGGCCAGAUGAGAGA	144	CACCAGGCCAGAUGAGAGA	144	UCUCUCAUCUGGCCUGGUG	882
GUGACAUAGCAGGAACUAC	145	GUGACAUAGCAGGAACUAC	145	GUAGUUCCUGCUAUGUCAC	883
AGCAGGAAGAUGGCCAGUA	146	AGCAGGAAGAUGGCCAGUA	146	UACUGGCCAUCUUCCUGCU	884
GAGAACCAAGGGGAAGUGA	147	GAGAACCAAGGGGAAGUGA	147	UCACUUCCCCUUGGUUCUC	885
AGUAUGGGCAAGCAGGGAG	148	AGUAUGGGCAAGCAGGGAG	148	CUCCCUGCUUGCCCAUACU	886
CCUACAAUCCCCAAAGUCA	149	CCUACAAUCCCCAAAGUCA	149	UGACUUUGGGGAUUGUAGG	887
CUACAAUCCCCAAAGUCAA	150	CUACAAUCCCCAAAGUCAA	150	UUGACUUUGGGGAUUGUAG	888
GCCUGUGUACCCACAGACC	151	GCCUGUGUACCCACAGACC	151	GGUCUGUGGGUACACAGGC	889
AGCAGAUGAUACAGUAUUA	152	AGCAGAUGAUACAGUAUUA	152	UAAUACUGUAUCAUCUGCU	890
AGAGAACCAAGGGGAAGUG	153	AGAGAACCAAGGGGAAGUG	153	CACUUCCCCUUGGUUCUCU	891
CCCUACAAUCCCCAAAGUC	154	CCCUACAAUCCCCAAAGUC	154	GACUUUGGGGAUUGUAGGG	892
UGACAUAGCAGGAACUACU	155	UGACAUAGCAGGAACUACU	155	AGUAGUUCCUGCUAUGUCA	893
UUAUCAGAAGGAGCCACCC	156	UUAUCAGAAGGAGCCACCC	156	GGGUGGCUCCUUCUGAUAA	894
AAGUGACAUAGCAGGAACU	157	AAGUGACAUAGCAGGAACU	157	AGUUCCUGCUAUGUCACUU	895
GCAGGAAGAUGGCCAGUAA	158	GCAGGAAGAUGGCCAGUAA	158	UUACUGGCCAUCUUCCUGC	896
UAGGCAUCUCCUAUGGCAG	159	UAGGCAUCUCCUAUGGCAG	159	CUGCCAUAGGAGAUGCCUA	897
CAAGGGGAAGUGACAUAGC	160	CAAGGGGAAGUGACAUAGC	160	GCUAUGUCACUUCCCCUUG	898
AAAGAGCAGAAGACAGUGG	161	AAAGAGCAGAAGACAGUGG	161	CCACUGUCUUCUGCUCUUU	899
CUCCUAUGGCAGGAAGAAG	162	CUCCUAUGGCAGGAAGAAG	162	CUUCUUCCUGCCAUAGGAG	900
UAUCAGAAGGAGCCACCCC	163	UAUCAGAAGGAGCCACCCC	163	GGGGUGGCUCCUUCUGAUA	901
AUUAUCAGAAGGAGCCACC	164	AUUAUCAGAAGGAGCCACC	164	GGUGGCUCCUUCUGAUAAU	902
AUGCCUGUGUACCCACAGA	165	AUGCCUGUGUACCCACAGA	165	UCUGUGGGUACACAGGCAU	903
AAAUUAGUAGAUUUCAGAG	166	AAAUUAGUAGAUUUCAGAG	166	CUCUGAAAUCUACUAAUUU	904
UGCAUAUAAGCAGCUGCUU	167	UGCAUAUAAGCAGCUGCUU	167	AAGCAGCUGCUUAUAUGCA	905
AAUUAGUAGAUUUCAGAGA	168	AAUUAGUAGAUUUCAGAGA	168	UCUCUGAAAUCUACUAAUU	906
GCAUCUCCUAUGGCAGGAA	169	GCAUCUCCUAUGGCAGGAA	169	UUCCUGCCAUAGGAGAUGC	907
AGAACCAAGGGGAAGUGAC	170	AGAACCAAGGGGAAGUGAC	170	GUCACUUCCCCUUGGUUCU	908
UCAAAAUUUUCGGGUUUAU	171	UCAAAAUUUUCGGGUUUAU	171	AUAAACCCGAAAAUUUUGA	909

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
CAGGGAUGGAAAGGAUCAC	172	CAGGGAUGGAAAGGAUCAC	172	GUGAUCCUUUCCAUCCCUG	910
GAAGGAGCCACCCCACAAG	173	GAAGGAGCCACCCCACAAG	173	CUUGUGGGGUGGCUCCUUC	911
AAUUUUCGGGUUUAUUACA	174	AAUUUUCGGGUUUAUUACA	174	UGUAAUAAACCCGAPAAUU	912
AGCAGGAAGCACUAUGGGC	175	AGCAGGAAGCACUAUGGGC	175	GCCCAUAGUGCUUCCUGCU	913
AUCAGAAGGAGCCACCCCA	176	AUCAGAAGGAGCCACCCCA	176	UGGGGUGGCUCCUUCUGAU	914
UGAGAGAACCAAGGGGAAG	177	UGAGAGAACCAAGGGGAAG	177	CUUCCCCUUGGUUCUCUCA	915
AAGGUGAAGGGGCAGUAGU	178	AAGGUGAAGGGGCAGUAGU	178	ACUACUGCCCCUUCACCUU	916
GAAAAAAUCAGUAACAGUA	179	GAAAAAAUCAGUAACAGUA	179	UACUGUUACUGAUUUUUUC	917
CAAUGAGGAAGCUGCAGAA	180	CAAUGAGGAAGCUGCAGAA	180	UUCUGCAGCUUCCUCAUUG	918
AGAUGAUACAGUAUUAGAA	181	AGAUGAUACAGUAUUAGAA	181	UUCUAAUACUGUAUCAUCU	919
UGAGGAAGCUGCAGAAUGG	182	UGAGGAAGCUGCAGAAUGG	182	CCAUUCUGCAGCUUCCUCA	920
UAUUAUGACCCAUCAAAAG	183	UAUUAUGACCCAUCAAAAG	183	CUUUUGAUGGGUCAUAAUA	921
UCACUCUUUGGCAACGACC	184	UCACUCUUUGGCAACGACC	184	GGUCGUUGCCAAAGAGUGA	922
UGGAGAAAAUUAGUAGAUU	185	UGGAGAAAAUUAGUAGAUU	185	AAUCUACUAAUUUUCUCCA	923
AGACAGGAUGAGGAUUAGA	186	AGACAGGAUGAGGAUUAGA	186	UCUAAUCCUCAUCCUGUCU	924
AAAGGUGAAGGGGCAGUAG	187	AAAGGUGAAGGGGCAGUAG	187	CUACUGCCCCUUCACCUUU	925
GGCAUCUCCUAUGGCAGGA	188	GGCAUCUCCUAUGGCAGGA	188	UCCUGCCAUAGGAGAUGCC	926
AAGGAGCCACCCCACAAGA	189	AAGGAGCCACCCCACAAGA	189	UCUUGUGGGGUGGCUCCUU	927
UAAAGCCAGGAAUGGAUGG	190	UAAAGCCAGGAAUGGAUGG	190	CCAUCCAUUCCUGGCUUUA	928
GGAGAAAAUUAGUAGAUUU	191	GGAGAAAAUUAGUAGAUUU	191	AAAUCUACUAAUUUUCUCC	929
AAGAGCAGAAGACAGUGGC	192	AAGAGCAGAAGACAGUGGC	192	GCCACUGUCUUCUGCUCUU	930
UCAGAAGGAGCCACCCCAC	193	UCAGAAGGAGCCACCCCAC	193	GUGGGGUGGCUCCUUCUGA	931
AGGCAUCUCCUAUGGCAGG	194	AGGCAUCUCCUAUGGCAGG	194	CCUGCCAUAGGAGAUGCCU	932
AGGGAUGGAAAGGAUCACC	195	AGGGAUGGAAAGGAUCACC	195	GGUGAUCCUUUCCAUCCCU	933
AGGAAGCUGCAGAAUGGGA	196	AGGAAGCUGCAGAAUGGGA	196	UCCCAUUCUGCAGCUUCCU	934
CUGCAUAUAAGCAGCUGCU	197	CUGCAUAUAAGCAGCUGCU	197	AGCAGCUGCUUAUAUGCAG	935
AAGGGGCAGUAGUAAUACA	198	AAGGGGCAGUAGUAAUACA	198	UGUAUUACUACUGCCCCUU	936
UUGACUAGCGGAGGCUAGA	199	UUGACUAGCGGAGGCUAGA	199	UCUAGCCUCCGCUAGUCAA	937
UAAAAGACACCAAGGAAGC	200	UAAAAGACACCAAGGAAGC	200	GCUUCCUUGGUGUCUUUUA	938
GAGGAAGCUGCAGAAUGGG	201	GAGGAAGCUGCAGAAUGGG	201	CCCAUUCUGCAGCUUCCUC	939
CAGCAGGAAGCACUAUGGG	202	CAGCAGGAAGCACUAUGGG	202	CCCAUAGUGCUUCCUGCUG	940
GGAGCCACCCCACAAGAUU	203	GGAGCCACCCCACAAGAUU	203	AAUCUUGUGGGGUGGCUCC	941
AUUAUGACCCAUCAAAAGA	204	AUUAUGACCCAUCAAAAGA	204	UCUUUUGAUGGGUCAUAAU	942
CAGAUGAUACAGUAUUAGA	205	CAGAUGAUACAGUAUUAGA	205	UCUAAUACUGUAUCAUCUG	943
AUGAGAGAACCAAGGGGAA	206	AUGAGAGAACCAAGGGGAA	206	UUCCCCUUGGUUCUCUCAU	944

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AUGAGGAAGCUGCAGAAUG	207	AUGAGGAAGCUGCAGAAUG	207	CAUUCUGCAGCUUCCUCAU	945
UGCCUGUGUACCCACAGAC	208	UGCCUGUGUACCCACAGAC	208	GUCUGUGGGUACACAGGCA	946
GAAGGGGCAGUAGUAAUAC	209	GAAGGGGCAGUAGUAAUAC	209	GUAUUACUACUGCCCCUUC	947
UCAGCAUUAUCAGAAGGAG	210	UCAGCAUUAUCAGAAGGAG	210	CUCCUUCUGAUAAUGCUGA	948
UUCAAAAUUUUCGGGUUUA	211	UUCAAAAUUUUCGGGUUUA	211	UAAACCCGAAAAUUUUGAA	949
UCUGGAAAGGUGAAGGGGC	212	UCUGGAAAGGUGAAGGGGC	212	GCCCCUUCACCUUUCCAGA	950
UUAGCAGGAAGAUGGCCAG	213	UUAGCAGGAAGAUGGCCAG	213	CUGGCCAUCUUCCUGCUAA	951
GAACCAAGGGGAAGUGACA	214	GAACCAAGGGGAAGUGACA	214	UGUCACUUCCCCUUGGUUC	952
AGAAGGAGCCACCCCACAA	215	AGAAGGAGCCACCCCACAA	215	UUGUGGGGUGGCUCCUUCU	953
AAUGAGGAAGCUGCAGAAU	216	AAUGAGGAAGCUGCAGAAU	216	AUUCUGCAGCUUCCUCAUU	954
AAGAAAAAAUCAGUAACAG	217	AAGAAAAAAUCAGUAACAG	217	CUGUUACUGAUUUUUUCUU	955
GGAAUUGGAGGUUUUAUCA	218	GGAAUUGGAGGUUUUAUCA	218	UGAUAAAACCUCCAAUUCC	956
UACAGUAUUAGUAGGACCU	219	UACAGUAUUAGUAGGACCU	219	AGGUCCUACUAAUACUGUA	957
CCAGGAAUGGAUGGCCCAA	220	CCAGGAAUGGAUGGCCCAA	220	UUGGGCCAUCCAUUCCUGG	958
UUCUAUGUAGAUGGGGCAG	221	UUCUAUGUAGAUGGGGCAG	221	CUGCCCCAUCUACAUAGAA	959
CAAAAUUUUCGGGUUUAUU	222	CAAAAUUUUCGGGUUUAUU	222	AAUAAACCCGAAAAUUUUG	960
UAGACAGGAUGAGGAUUAG	223	UAGACAGGAUGAGGAUUAG	223	CUAAUCCUCAUCCUGUCUA	961
UGACAGAAGAAAAAAUAAA	224	UGACAGAAGAAAAAAUAAA	224	UUUAUUUUUUCUUCUGUCA	962
UUUAUUACAGGGACAGCAG	225	UUUAUUACAGGGACAGCAG	225	CUGCUGUCCCUGUAAUAAA	963
GGGUUUAUUACAGGGACAG	226	GGGUUUAUUACAGGGACAG	226	CUGUCCCUGUAAUAAACCC	964
AGAUGGAACAAGCCCCAGA	227	AGAUGGAACAAGCCCCAGA	227	UCUGGGGCUUGUUCCAUCU	965
CUAGCGGAGGCUAGAAGGA	228	CUAGCGGAGGCUAGAAGGA	228	UCCUUCUAGCCUCCGCUAG	966
UGACUAGCGGAGGCUAGAA	229	UGACUAGCGGAGGCUAGAA	229	UUCUAGCCUCCGCUAGUCA	967
GACAUAAUAGCAACAGACA	230	GACAUAAUAGCAACAGACA	230	UGUCUGUUGCUAUUAUGUC	968
GGUUUAUUACAGGGACAGC	231	GGUUUAUUACAGGGACAGC	231	GCUGUCCCUGUAAUAAACC	969
GCAGGUGAUGAUUGUGUGG	232	GCAGGUGAUGAUUGUGUGG	232	CCACACAAUCAUCACCUGC	970
AUGGCAGGAAGAAGCGGAG	233	AUGGCAGGAAGAAGCGGAG	233	CUCCGCUUCUUCCUGCCAU	971
AGGUGAUGAUUGUGUGGCA	234	AGGUGAUGAUUGUGUGGCA	234	UGCCACACAAUCAUCACCU	972
CCACCCCACAAGAUUUAAA	235	CCACCCCACAAGAUUUAAA	235	UUUAAAUCUUGUGGGGUGG	973
GUAAAAAAUUGGAUGACAG	236	GUAAAAAAUUGGAUGACAG	236	CUGUCAUCCAAUUUUUUAC	974
AUAAUAGCAACAGACAUAC	237	AUAAUAGCAACAGACAUAC	237	GUAUGUCUGUUGCUAUUAU	975
GCAUAUAAGCAGCUGCUUU	238	GCAUAUAAGCAGCUGCUUU	238	AAAGCAGCUGCUUAUAUGC	976
GGCAGGUGAUGAUUGUGUG	239	GGCAGGUGAUGAUUGUGUG	239	CACACAAUCAUCACCUGCC	977
AUGAUACAGUAUUAGAAGA	240	AUGAUACAGUAUUAGAAGA	240	UCUUCUAAUACUGUAUCAU	978
GAUGGCAGGUGAUGAUUGU	241	GAUGGCAGGUGAUGAUUGU	241	ACAAUCAUCACCUGCCAUC	979
CAUAAUAGCAACAGACAUA	242	CAUAAUAGCAACAGACAUA	242	UAUGUCUGUUGCUAUUAUG	980

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AAAAUUUUCGGGUUUAUUA	243	AAAAUUUUCGGGUUUAUUA	243	UAAUAAACCCGAAAAUUUU	981
ACAUAAUAGCAACAGACAU	244	ACAUAAUAGCAACAGACAU	244	AUGUCUGUUGCUAUUAUGU	982
AUUUCAAAAAUUGGGCCUG	245	AUUUCAAAAAUUGGGCCUG	245	CAGGCCCAAUUUUUGAAAU	983
CUGGAAAGGUGAAGGGGCA	246	CUGGAAAGGUGAAGGGGCA	246	UGCCCCUUCACCUUUCCAG	984
AAAACAGAUGGCAGGUGAU	247	AAAACAGAUGGCAGGUGAU	247	AUCACCUGCCAUCUGUUUU	985
UUUCAAAAAUUGGGCCUGA	248	UUUCAAAAAUUGGGCCUGA	248	UCAGGCCCAAUUUUUGAAA	986
GAGAGAACCAAGGGGAAGU	249	GAGAGAACCAAGGGGAAGU	249	ACUUCCCCUUGGUUCUCUC	987
CUCUGGAAAGGUGAAGGGG	250	CUCUGGAAAGGUGAAGGGG	250	CCCCUUCACCUUUCCAGAG	988
AUUAGCAGGAAGAUGGCCA	251	AUUAGCAGGAAGAUGGCCA	251	UGGCCAUCUUCCUGCUAAU	989
GAGCCACCCCACAAGAUUU	252	GAGCCACCCCACAAGAUUU	252	AAAUCUUGUGGGGUGGCUC	990
CAUAGCAGGAACUACUAGU	253	CAUAGCAGGAACUACUAGU	253	ACUAGUAGUUCCUGCUAUG	991
UUUUAAAAGAAAAGGGGGG	254	UUUUAAAAGAAAAGGGGGG	254	CCCCCCUUUUCUUUUAAAA	992
GCGGAGGCUAGAAGGAGAG	255	GCGGAGGCUAGAAGGAGAG	255	CUCUCCUUCUAGCCUCCGC	993
CAGUAUUAGUAGGACCUAC	256	CAGUAUUAGUAGGACCUAC	256	GUAGGUCCUACUAAUACUG	994
AGGGGGAAUUGGAGGUUUU	257	AGGGGGAAUUGGAGGUUUU	257	AAAACCUCCAAUUCCCCCU	995
ACAGUAUUAGUAGGACCUA	258	ACAGUAUUAGUAGGACCUA	258	UAGGUCCUACUAAUACUGU	996
GACUAGCGGAGGCUAGAAG	259	GACUAGCGGAGGCUAGAAG	259	CUUCUAGCCUCCGCUAGUC	997
GUUUAUUACAGGGACAGCA	260	GUUUAUUACAGGGACAGCA	260	UGCUGUCCCUGUAAUAAAC	998
CAGGUGAUGAUUGUGUGGC	261	CAGGUGAUGAUUGUGUGGC	261	GCCACACAAUCAUCACCUG	999
AGCGGAGGCUAGAAGGAGA	262	AGCGGAGGCUAGAAGGAGA	262	UCUCCUUCUAGCCUCCGCU	1000
UCUAUGUAGAUGGGGCAGC	263	UCUAUGUAGAUGGGGCAGC	263	GCUGCCCCAUCUACAUAGA	1001
UAAAAAAUUGGAUGACAGA	264	UAAAAAAUUGGAUGACAGA	264	UCUGUCAUCCAAUUUUUUA	1002
GCAGCAGGAAGCACUAUGG	265	GCAGCAGGAAGCACUAUGG	265	CCAUAGUGCUUCCUGCUGC	1003
UUAUUACAGGGACAGCAGA	266	UUAUUACAGGGACAGCAGA	266	UCUGCUGUCCCUGUAAUAA	1004
AAACAGAUGGCAGGUGAUG	267	AAACAGAUGGCAGGUGAUG	267	CAUCACCUGCCAUCUGUUU	1005
AUUCAAAAUUUUCGGGUUU	268	AUUCAAAAUUUUCGGGUUU	268	AAACCCGAAAAUUUUGAAU	1006
GGGGAAUUGGAGGUUUUAU	269	GGGGAAUUGGAGGUUUUAU	269	AUAAAACCUCCAAUUCCCC	1007
GCCACCCCACAAGAUUUAA	270	GCCACCCCACAAGAUUUAA	270	UUAAAUCUUGUGGGGUGGC	1008
GAUGAUACAGUAUUAGAAG	271	GAUGAUACAGUAUUAGAAG	271	CUUCUAAUACUGUAUCAUC	1009
UAAUAGCAACAGACAUACA	272	UAAUAGCAACAGACAUACA	272	UGUAUGUCUGUUGCUAUUA	1010
GAGGCUAGAAGGAGAGAGA	273	GAGGCUAGAAGGAGAGAGA	273	UCUCUCUCCUUCUAGCCUC	1011
GUACAGUAUUAGUAGGACC	274	GUACAGUAUUAGUAGGACC	274	GGUCCUACUAAUACUGUAC	1012
UAGCGGAGGCUAGAAGGAG	275	UAGCGGAGGCUAGAAGGAG	275	CUCCUUCUAGCCUCCGCUA	1013
CGGAGGCUAGAAGGAGAGA	276	CGGAGGCUAGAAGGAGAGA	276	UCUCUCCUUCUAGCCUCCG	1014
GGUACAGUAUUAGUAGGAC	277	GGUACAGUAUUAGUAGGAC	277	GUCCUACUAAUACUGUACC	1015

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HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AAAUUUUCGGGUUUAUUAC	278	AAAUUUUCGGGUUUAUUAC	278	GUAAUAAACCCGAAAAUUU	1016
AGCAGCAGGAAGCACUAUG	279	AGCAGCAGGAAGCACUAUG	279	CAUAGUGCUUCCUGCUGCU	1017
AGCCACCCCACAAGAUUUA	280	AGCCACCCCACAAGAUUUA	280	UAAAUCUUGUGGGGUGGCU	1018
AACCAAGGGGPAGUGACAU	281	AACCAAGGGGAAGUGACAU	281	AUGUCACUUCCCCUUGGUU	1019
AAGGGGAAGUGACAUAGCA	282	AAGGGGAAGUGACAUAGCA	282	UGCUAUGUCACUUCCCCUU	1020
UUAAAGCCAGGAAUGGAUG	283	UUAAAGCCAGGAAUGGAUG	283	CAUCCAUUCCUGGCUUUAA	1021
ACUAGCGGAGGCUAGAAGG	284	ACUAGCGGAGGCUAGAAGG	284	CCUUCUAGCCUCCGCUAGU	1022
UAGGUACAGUAUUAGUAGG	285	UAGGUACAGUAUUAGUAGG	285	CCUACUAAUACUGUACCUA	1023
GGGGGAAUUGGAGGUUUUA	286	GGGGGAAUUGGAGGUUUUA	286	UAAAACCUCCAAUUCCCCC	1024
AGAUGGCAGGUGAUGAUUG	287	AGAUGGCAGGUGAUGAUUG	287	CAAUCAUCACCUGCCAUCU	1025
UUAAACAAUGGCCAUUGAC	288	UUAAACAAUGGCCAUUGAC	288	GUCAAUGGCCAUUGUUUAA	1026
UGGCAGGUGAUGAUUGUGU	289	UGGCAGGUGAUGAUUGUGU	289	ACACAAUCAUCACCUGCCA	1027
UAAAAUUAGCAGGAAGAUG	290	UAAAAUUAGCAGGAAGAUG	290	CAUCUUCCUGCUAAUUUUA	1028
AGGAGCCACCCCACAAGAU	291	AGGAGCCACCCCACAAGAU	291	AUCUUGUGGGGUGGCUCCU	1029
GUAUUAGUAGGACCUACAC	292	GUAUUAGUAGGACCUACAC	292	GUGUAGGUCCUACUAAUAC	1030
AAUCCCCAAAGUCAAGGAG	293	AAUCCCCAAAGUCAAGGAG	293	CUCCUUGACUUUGGGGAUU	1031
CCAGGCCAGAUGAGAGAAC	294	CCAGGCCAGAUGAGAGAAC	294	GUUCUCUCAUCUGGCCUGG	1032
CCAUUGACAGAAGAAAAAA	295	CCAUUGACAGAAGAAAAAA	295	UUUUUUCUUCUGUCAAUGG	1033
CAGAUGGCAGGUGAUGAUU	296	CAGAUGGCAGGUGAUGAUU	296	AAUCAUCACCUGCCAUCUG	1034
CAGAUGAGAGAACCAAGGG	297	CAGAUGAGAGAACCAAGGG	297	CCCUUGGUUCUCUCAUCUG	1035
GCCAUUGACAGAAGAAAAA	298	GCCAUUGACAGAAGAAAAA	298	UUUUUCUUCUGUCAAUGGC	1036
UAUUAGUAGGACCUACACC	299	UAUUAGUAGGACCUACACC	299	GGUGUAGGUCCUACUAAUA	1037
UCUCGACGCAGGACUCGGC	300	UCUCGACGCAGGACUCGGC	300	GCCGAGUCCUGCGUCGAGA	1038
AGAUGAGAGAACCAAGGGG	301	AGAUGAGAGAACCAAGGGG	301	CCCCUUGGUUCUCUCAUCU	1039
AUCCCCAAAGUCAAGGAGU	302	AUCCCCAAAGUCAAGGAGU	302	ACUCCUUGACUUUGGGGAU	1040
AAUUAGCAGGAAGAUGGCC	303	AAUUAGCAGGAAGAUGGCC	303	GGCCAUCUUCCUGCUAAUU	1041
GGGAAUUGGAGGUUUUAUC	304	GGGAAUUGGAGGUUUUAUC	304	GAUAAAACCUCCAAUUCCC	1042
CUCGACGCAGGACUCGGCU	305	CUCGACGCAGGACUCGGCU	305	AGCCGAGUCCUGCGUCGAG	1043
AUGGCCAUUGACAGAAGAA	306	AUGGCCAUUGACAGAAGAA	306	UUCUUCUGUCAAUGGCCAU	1044
AAAAUUAGCAGGAAGAUGG	307	AAAAUUAGCAGGAAGAUGG	307	CCAUCUUCCUGCUAAUUUU	1045
ACGCAGGACUCGGCUUGCU	308	ACGCAGGACUCGGCUUGCU	308	AGCAAGCCGAGUCCUGCGU	1046
UAAACAAUGGCCAUUGACA	309	UAAACAAUGGCCAUUGACA	309	UGUCAAUGGCCAUUGUUUA	1047
GAUGGAACAAGCCCCAGAA	310	GAUGGAACAAGCCCCAGAA	310	UUCUGGGGCUUGUUCCAUC	1048
AAUGAACAAGUAGAUAAAU	311	AAUGAACAAGUAGAUAAAU	311	AUUUAUCUACUUGUUCAUU	1049
AUUGGAGGUUUUAUCAAAG	312	AUUGGAGGUUUUAUCAAAG	312	CUUUGAUAAAACCUCCAAU	1050
AGGCUAGAAGGAGAGAGAU	313	AGGCUAGAAGGAGAGAGAU	313	AUCUCUCUCCUUCUAGCCU	1051

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AGAUGGGUGCGAGAGCGUC	314	AGAUGGGUGCGAGAGCGUC	314	GACGCUCUCGCACCCAUCU	1052
AGGUACAGUAUUAGUAGGA	315	AGGUACAGUAUUAGUAGGA	315	UCCUACUAAUACUGUACCU	1053
GGAGGCUAGAAGGAGAGAG	316	GGAGGCUAGAAGGAGAGAG	316	CUCUCUCCUUCUAGCCUCC	1054
CAGGACAUAACAAGGUAGG	317	CAGGACAUAACAAGGUAGG	317	CCUACCUUGUUAUGUCCUG	1055
AGUAUUAGUAGGACCUACA	318	AGUAUUAGUAGGACCUACA	318	UGUAGGUCCUACUAAUACU	1056
UUGACAGAAGAAAAAAUAA	319	UUGACAGAAGAAAAAAUAA	319	UUAUUUUUUCUUCUGUCAA	1057
UGGAGAAGUGAAUUAUAUA	320	UGGAGAAGUGAAUUAUAUA	320	UAUAUAAUUCACUUCUCCA	1058
CUCUCGACGCAGGACUCGG	321	CUCUCGACGCAGGACUCGG	321	CCGAGUCCUGCGUCGAGAG	1059
AUGAACAAGUAGAUAAAUU	322	AUGAACAAGUAGAUAAAUU	322	AAUUUAUCUACUUGUUCAU	1060
UGGCCAUUGACAGAAGAAA	323	UGGCCAUUGACAGAAGAAA	323	UUUCUUCUGUCAAUGGCCA	1061
AUACCCAUGUUUUCAGCAU	324	AUACCCAUGUUUUCAGCAU	324	AUGCUGAAAACAUGGGUAU	1062
UUUAAAAGAAAAGGGGGGA	325	UUUAAAAGAAAAGGGGGGA	325	UCCCCCCUUUUCUUUUAAA	1063
CGACGCAGGACUCGGCUUG	326	CGACGCAGGACUCGGCUUG	326	CAAGCCGAGUCCUGCGUCG	1064
AUUGACAGAAGAAAAAAUA	327	AUUGACAGAAGAAAAAAUA	327	UAUUUUUUCUUCUGUCAAU	1065
CUAGAAGGAGAGAGAUGGG	328	CUAGAAGGAGAGAGAUGGG	328	CCCAUCUCUCUCCUUCUAG	1066
UGGCAGGAAGAAGCGGAGA	329	UGGCAGGAAGAAGCGGAGA	329	UCUCCGCUUCUUCCUGCCA	1067
CAAUCCCCAAAGUCAAGGA	330	CAAUCCCCAAAGUCAAGGA	330	UCCUUGACUUUGGGGAUUG	1068
AAAUUCAAAAUUUUCGGGU	331	AAAUUCAAAAUUUUCGGGU	331	ACCCGAAAAUUUUGAAUUU	1069
GAAUUGGAGGUUUUAUCAA	332	GAAUUGGAGGUUUUAUCAA	332	UUGAUAAAACCUCCAAUUC	1070
GACGCAGGACUCGGCUUGC	333	GACGCAGGACUCGGCUUGC	333	GCAAGCCGAGUCCUGCGUC	1071
UUUGACUAGCGGAGGCUAG	334	UUUGACUAGCGGAGGCUAG	334	CUAGCCUCCGCUAGUCAAA	1072
AUAGGUACAGUAUUAGUAG	335	AUAGGUACAGUAUUAGUAG	335	CUACUAAUACUGUACCUAU	1073
GGCUAGAAGGAGAGAGAUG	336	GGCUAGAAGGAGAGAGAUG	336	CAUCUCUCUCCUUCUAGCC	1074
ACCAGGCCAGAUGAGAGAA	337	ACCAGGCCAGAUGAGAGAA	337	UUCUCUCAUCUGGCCUGGU	1075
GAUGAGAGAACCAAGGGGA	338	GAUGAGAGAACCAAGGGGA	338	UCCCCUUGGUUCUCUCAUC	1076
GGAGCAGCAGGAAGCACUA	339	GGAGCAGCAGGAAGCACUA	339	UAGUGCUUCCUGCUGCUCC	1077
UCUCUCGACGCAGGACUCG	340	UCUCUCGACGCAGGACUCG	340	CGAGUCCUGCGUCGAGAGA	1078
UCCCUACAAUCCCCAAAGU	341	UCCCUACAAUCCCCAAAGU	341	ACUUUGGGGAUUGUAGGGA	1079
UUGGAGGUUUUAUCAAAGU	342	UUGGAGGUUUUAUCAAAGU	342	ACUUUGAUAAAACCUCCAA	1080
ACUGUACCAGUAAAAUUAA	343	ACUGUACCAGUAAAAUUAA	343	UUAAUUUUACUGGUACAGU	1081
AUGGCAGGUGAUGAUUGUG	344	AUGGCAGGUGAUGAUUGUG	344	CACAAUCAUCACCUGCCAU	1082
GAGGAAAUGAACAAGUAGA	345	GAGGAAAUGAACAAGUAGA	345	UCUACUUGUUCAUUUCCUC	1083
AGACAUAAUAGCAACAGAC	346	AGACAUAAUAGCAACAGAC	346	GUCUGUUGCUAUUAUGUCU	1084
AAAUUAGCAGGAAGAUGGC	347	AAAUUAGCAGGAAGAUGGC	347	GCCAUCUUCCUGCUAAUUU	1085
UUGGAGAAGUGAAUUAUAU	348	UUGGAGAAGUGAAUUAUAU	348	AUAUAAUUCACUUCUCCAA	1086

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
UCGACGCAGGACUCGGCUU	349	UCGACGCAGGACUCGGCUU	349	AAGCCGAGUCCUGCGUCGA	1087
AAAAUUCAAAAUUUUCGGG	350	AAAAUUCAAAAUUUUCGGG	350	CCCGAAAAUUUUGAAUUUU	1088
CAGGCCAGAUGAGAGAACC	351	CAGGCCAGAUGAGAGAACC	351	GGUUCUCUCAUCUGGCCUG	1089
UACCCAUGUUUUCAGCAUU	352	UACCCAUGUUUUCAGCAUU	352	AAUGCUGAAAACAUGGGUA	1090
ACACAUGCCUGUGUACCCA	353	ACACAUGCCUGUGUACCCA	353	UGGGUACACAGGCAUGUGU	1091
GGCCAUUGACAGAAGAAAA	354	GGCCAUUGACAGAAGAAAA	354	UUUUCUUCUGUCAAUGGCC	1092
GAGCAGCAGGAAGCACUAU	355	GAGCAGCAGGAAGCACUAU	355	AUAGUGCUUCCUGCUGCUC	1093
CUGUACCAGUAAAAUUAAA	356	CUGUACCAGUAAAAUUAAA	356	UUUAAUUUUACUGGUACAG	1094
GAAAUGAUGACAGCAUGUC	357	GAAAUGAUGACAGCAUGUC	357	GACAUGCUGUCAUCAUUUC	1095
CAUUGACAGAAGAAAAAAU	358	CAUUGACAGAAGAAAAAAU	358	AUUUUUUCUUCUGUCAAUG	1096
AAAUGAUGACAGCAUGUCA	359	AAAUGAUGACAGCAUGUCA	359	UGACAUGCUGUCAUCAUUU	1097
GCUAGAAGGAGAGAGAUGG	360	GCUAGAAGGAGAGAGAUGG	360	CCAUCUCUCUCCUUCUAGC	1098
UAGGGAUUAUGGAAAACAG	361	UAGGGAUUAUGGAAAACAG	361	CUGUUUUCCAUAAUCCCUA	1099
GAAAAUUAGUAGAUUUCAG	362	GAAAAUUAGUAGAUUUCAG	362	CUGAAAUCUACUAAUUUUC	1100
CUACACCUGUCAACAUAAU	363	CUACACCUGUCAACAUAAU	363	AUUAUGUUGACAGGUGUAG	1101
ACAGAUGGCAGGUGAUGAU	364	ACAGAUGGCAGGUGAUGAU	364	AUCAUCACCUGCCAUCUGU	1102
CCACAGGGAUGGAAAGGAU	365	CCACAGGGAUGGAAAGGAU	365	AUCCUUUCCAUCCCUGUGG	1103
UUAGGGAUUAUGGAAAACA	366	UUAGGGAUUAUGGAAAACA	366	UGUUUUCCAUAAUCCCUAA	1104
AGAUGCUGCAUAUAAGCAG	367	AGAUGCUGCAUAUAAGCAG	367	CUGCUUAUAUGCAGCAUCU	1105
AAUAGCAACAGACAUACAA	368	AAUAGCAACAGACAUACAA	368	UUGUAUGUCUGUUGCUAUU	1106
AAUUCAAAAUUUUCGGGUU	369	AAUUCAAAAUUUUCGGGUU	369	AACCCGAAAAUUUUGAAUU	1107
CAGACUCACAAUAUGCAUU	370	CAGACUCACAAUAUGCAUU	370	AAUGCAUAUUGUGAGUCUG	1108
UAUGCAUUAGGAAUCAUUC	371	UAUGCAUUAGGAAUCAUUC	371	GAAUGAUUCCUAAUGCAUA	1109
UACACCUGUCAACAUAAUU	372	UACACCUGUCAACAUAAUU	372	AAUUAUGUUGACAGGUGUA	1110
UGGAGGAAAUGAACAAGUA	373	UGGAGGAAAUGAACAAGUA	373	UACUUGUUCAUUUCCUCCA	1111
ACCAAGGGGAAGUGACAUA	374	ACCAAGGGGAAGUGACAUA	374	UAUGUCACUUCCCCUUGGU	1112
GAGAUGGGUGCGAGAGCGU	375	GAGAUGGGUGCGAGAGCGU	375	ACGCUCUCGCACCCAUCUC	1113
UAUAGGUACAGUAUUAGUA	376	UAUAGGUACAGUAUUAGUA	376	UACUAAUACUGUACCUAUA	1114
AUUAGGGAUUAUGGAAAAC	377	AUUAGGGAUUAUGGAAAAC	377	GUUUUCCAUAAUCCCUAAU	1115
UGGCUGUGGAAAGAUACCU	378	UGGCUGUGGAAAGAUACCU	378	AGGUAUCUUUCCACAGCCA	1116
GAGAGAUGGGUGCGAGAGC	379	GAGAGAUGGGUGCGAGAGC	379	GCUCUCGCACCCAUCUCUC	1117
CCUACACCUGUCAACAUAA	380	CCUACACCUGUCAACAUAA	380	UUAUGUUGACAGGUGUAGG	1118
CAGCAGUACAAAUGGCAGU	381	CAGCAGUACAAAUGGCAGU	381	ACUGCCAUUUGUACUGCUG	1119
GGCUGUGGAAAGAUACCUA	382	GGCUGUGGAAAGAUACCUA	382	UAGGUAUCUUUCCACAGCC	1120
AGAAAAUUAGUAGAUUUCA	383	AGAAAAUUAGUAGAUUUCA	383	UGAAAUCUACUAAUUUUCU	1121
GCCACCUUUGCCUAGUGUU	384	GCCACCUUUGCCUAGUGUU	384	AACACUAGGCAAAGGUGGC	1122

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GAUGCUGCAUAUAAGCAGC	385	GAUGCUGCAUAUAAGCAGC	385	GCUGCUUAUAUGCAGCAUC	1123
GCUAUAGGUACAGUAUUAG	386	GCUAUAGGUACAGUAUUAG	386	CUAAUACUGUACCUAUAGC	1124
AACAGAUGGCAGGUGAUGA	387	AACAGAUGGCAGGUGAUGA	387	UCAUCACCUGCCAUCUGUU	1125
AUCACUCUUUGGCPACGAC	388	AUCACUCUUUGGCAACGAC	388	GUCGUUGCCAAAGAGUGAU	1126
ACAUGCCUGUGUACCCACA	389	ACAUGCCUGUGUACCCACA	389	UGUGGGUACACAGGCAUGU	1127
ACAGCAGUACAAAUGGCAG	390	ACAGCAGUACAAAUGGCAG	390	CUGCCAUUUGUACUGCUGU	1128
AUGCAUUAGGAAUCAUUCA	391	AUGCAUUAGGAAUCAUUCA	391	UGAAUGAUUCCUAAUGCAU	1129
AAUUGGAGGUUUUAUCAAA	392	AAUUGGAGGUUUUAUCAAA	392	UUUGAUAAAACCUCCAAUU	1130
UUGGAGGAAAUGAACAAGU	393	UUGGAGGAAAUGAACAAGU	393	ACUUGUUCAUUUCCUCCAA	1131
AUUGGAGGAAAUGAACAAG	394	AUUGGAGGAAAUGAACAAG	394	CUUGUUCAUUUCCUCCAAU	1132
AAAAAUUCAAAAUUUUCGG	395	AAAAAUUCAAAAUUUUCGG	395	CCGAAAAUUUUGAAUUUUU	1133
AGGUGAAGGGGCAGUAGUA	396	AGGUGAAGGGGCAGUAGUA	396	UACUACUGCCCCUUCACCU	1134
CUAUAGGUACAGUAUUAGU	397	CUAUAGGUACAGUAUUAGU	397	ACUAAUACUGUACCUAUAG	1135
AUUAAAGCCAGGAAUGGAU	398	AUUAAAGCCAGGAAUGGAU	398	AUCCAUUCCUGGCUUUAAU	1136
GGAGGAAAUGAACAAGUAG	399	GGAGGAAAUGAACAAGUAG	399	CUACUUGUUCAUUUCCUCC	1137
AGCAGUACAAAUGGCAGUA	400	AGCAGUACAAAUGGCAGUA	400	UACUGCCAUUUGUACUGCU	1138
AUCAGUACAAUGUGCUUCC	401	AUCAGUACAAUGUGCUUCC	401	GGAAGCACAUUGUACUGAU	1139
UAUGGGGUACCUGUGUGGA	402	UAUGGGGUACCUGUGUGGA	402	UCCACACAGGUACCCCAUA	1140
AGAGAUGGGUGCGAGAGCG	403	AGAGAUGGGUGCGAGAGCG	403	CGCUCUCGCACCCAUCUCU	1141
GGUGAAGGGGCAGUAGUAA	404	GGUGAAGGGGCAGUAGUAA	404	UUACUACUGCCCCUUCACC	1142
GUGAAGGGGCAGUAGUAAU	405	GUGAAGGGGCAGUAGUAAU	405	AUUACUACUGCCCCUUCAC	1143
CGCAGGACUCGGCUUGCUG	406	CGCAGGACUCGGCUUGCUG	406	CAGCAAGCCGAGUCCUGCG	1144
CACAUGCCUGUGUACCCAC	407	CACAUGCCUGUGUACCCAC	407	GUGGGUACACAGGCAUGUG	1145
GAGAGAGAUGGGUGCGAGA	408	GAGAGAGAUGGGUGCGAGA	408	UCUCGCACCCAUCUCUCUC	1146
UAGAAGGAGAGAGAUGGGU	409	UAGAAGGAGAGAGAUGGGU	409	ACCCAUCUCUCUCCUUCUA	1147
CACAGGGAUGGAAAGGAUC	410	CACAGGGAUGGAAAGGAUC	410	GAUCCUUUCCAUCCCUGUG	1148
GGCAGGAAGAAGCGGAGAC	411	GGCAGGAAGAAGCGGAGAC	411	GUCUCCGCUUCUUCCUGCC	1149
UCCCCAAAGUCAAGGAGUA	412	UCCCCAAAGUCAAGGAGUA	412	UACUCCUUGACUUUGGGGA	1150
CCUGUCAACAUAAUUGGAA	413	CCUGUCAACAUAAUUGGAA	413	UUCCAAUUAUGUUGACAGG	1151
UAUCAGUACAAUGUGCUUC	414	UAUCAGUACAAUGUGCUUC	414	GAAGCACAUUGUACUGAUA	1152
UGAAGGGGCAGUAGUAAUA	415	UGAAGGGGCAGUAGUAAUA	415	UAUUACUACUGCCCCUUCA	1153
CUCAGAUGCUGCAUAUAAG	416	CUCAGAUGCUGCAUAUAAG	416	CUUAUAUGCAGCAUCUGAG	1154
ACAGGGAUGGAAAGGAUCA	417	ACAGGGAUGGAAAGGAUCA	417	UGAUCCUUUCCAUCCCUGU	1155
AAGAAAAGGGGGGAUUGGG	418	AAGAAAAGGGGGGAUUGGG	418	CCCAAUCCCCCCUUUUCUU	1156
UCAUUAGGGAUUAUGGAAA	419	UCAUUAGGGAUUAUGGAAA	419	UUUCCAUAAUCCCUAAUGA	1157

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GAAGGAGAGAGAUGGGUGC	420	GAAGGAGAGAGAUGGGUGC	420	GCACCCAUCUCUCUCCUUC	1158
GUUAAACAAUGGCCAUUGA	421	GUUAAACAAUGGCCAUUGA	421	UCAAUGGCCAUUGUUUAAC	1159
AUGGACAAGUAGACUGUAG	422	AUGGACAAGUAGACUGUAG	422	CUACAGUCUACUUGUCCAU	1160
UAGUAGAUUUCAGAGAACU	423	UAGUAGAUUUCAGAGAACU	423	AGUUCUCUGAAAUCUACUA	1161
CUGUCAACAUAAUUGGAAG	424	CUGUCAACAUAAUUGGAAG	424	CUUCCAAUUAUGUUGACAG	1162
GGGGCAGUAGUAAUACAAG	425	GGGGCAGUAGUAAUACAAG	425	CUUGUAUUACUACUGCCCC	1163
CAUUAGGGAUUAUGGAAAA	426	CAUUAGGGAUUAUGGAAAA	426	UUUUCCAUAAUCCCUAAUG	1164
GAACUACUAGUACCCUUCA	427	GAACUACUAGUACCCUUCA	427	UGAAGGGUACUAGUAGUUC	1165
GCAGGAAGCACUAUGGGCG	428	GCAGGAAGCACUAUGGGCG	428	CGCCCAUAGUGCUUCCUGC	1166
AAGGAGAGAGAUGGGUGCG	429	AAGGAGAGAGAUGGGUGCG	429	CGCACCCAUCUCUCUCCUU	1167
CAGGAAUGGAUGGCCCAAA	430	CAGGAAUGGAUGGCCCAAA	430	UUUGGGCCAUCCAUUCCUG	1168
GGAAAUGAACAAGUAGAUA	431	GGAAAUGAACAAGUAGAUA	431	UAUCUACUUGUUCAUUUCC	1169
AAAAGACACCAAGGAAGCU	432	AAAAGACACCAAGGAAGCU	432	AGCUUCCUUGGUGUCUUUU	1170
AUCAUUCAAGCACAACCAG	433	AUCAUUCAAGCACAACCAG	433	CUGGUUGUGCUUGAAUGAU	1171
AACAAGUAGAUAAAUUAGU	434	AACAAGUAGAUAAAUUAGU	434	ACUAAUUUAUCUACUUGUU	1172
AGGAAAUGAACAAGUAGAU	435	AGGAAAUGAACAAGUAGAU	435	AUCUACUUGUUCAUUUCCU	1173
GCAGGACUCGGCUUGCUGA	436	GCAGGACUCGGCUUGCUGA	436	UCAGCAAGCCGAGUCCUGC	1174
GAAUCAUUCAAGCACAACC	437	GAAUCAUUCAAGCACAACC	437	GGUUGUGCUUGAAUGAUUC	1175
CCUCAGAUGCUGCAUAUAA	438	CCUCAGAUGCUGCAUAUAA	438	UUAUAUGCAGCAUCUGAGG	1176
GAUGGAAAGGAUCACCAGC	439	GAUGGAAAGGAUCACCAGC	439	GCUGGUGAUCCUUUCCAUC	1177
AGGAGAGAGAUGGGUGCGA	440	AGGAGAGAGAUGGGUGCGA	440	UCGCACCCAUCUCUCUCCU	1178
CAUGGACAAGUAGACUGUA	441	CAUGGACAAGUAGACUGUA	441	UACAGUCUACUUGUCCAUG	1179
UCAGAUGCUGCAUAUAAGC	442	UCAGAUGCUGCAUAUAAGC	442	GCUUAUAUGCAGCAUCUGA	1180
AUGGAGAAAAUUAGUAGAU	443	AUGGAGAAAAUUAGUAGAU	443	AUCUACUAAUUUUCUCCAU	1181
GAGAAAAUUAGUAGAUUUC	444	GAGAAAAUUAGUAGAUUUC	444	GAAAUCUACUAAUUUUCUC	1182
AUGACAGCAUGUCAGGGAG	445	AUGACAGCAUGUCAGGGAG	445	CUCCCUGACAUGCUGUCAU	1183
AGGCCAGAUGAGAGAACCA	446	AGGCCAGAUGAGAGAACCA	446	UGGUUCUCUCAUCUGGCCU	1184
AGAGAGAUGGGUGCGAGAG	447	AGAGAGAUGGGUGCGAGAG	447	CUCUCGCACCCAUCUCUCU	1185
ACCCAUGUUUUCAGCAUUA	448	ACCCAUGUUUUCAGCAUUA	448	UAAUGCUGAAAACAUGGGU	1186
GAUGACAGCAUGUCAGGGA	449	GAUGACAGCAUGUCAGGGA	449	UCCCUGACAUGGUGUCAUC	1187
AGCCAGGAAUGGAUGGCCC	450	AGCCAGGAAUGGAUGGCCC	450	GGGCCAUCCAUUCCUGGCU	1188
UGAUGACAGCAUGUCAGGG	451	UGAUGACAGCAUGUCAGGG	451	CCCUGACAUGCUGUCAUCA	1189
CAGGAAGCACUAUGGGCGC	452	CAGGAAGCACUAUGGGCGC	452	GCGCCCAUAGUGCUUCCUG	1190
ACAGACUCACAAUAUGCAU	453	ACAGACUCACAAUAUGCAU	453	AUGCAUAUUGUGAGUCUGU	1191
UGGAGGUUUUAUCAAAGUA	454	UGGAGGUUUUAUCAAAGUA	454	UACUUUGAUAAAACCUCCA	1192
AAGCCAGGAAUGGAUGGCC	455	AAGCCAGGAAUGGAUGGCC	455	GGCCAUCCAUUCCUGGCUU	1193

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
UUUUGACUAGCGGAGGCUA	456	UUUUGACUAGCGGAGGCUA	456	UAGCCUCCGCUAGUCAAAA	1194
CAGAUGCUGCAUAUAAGCA	457	CAGAUGCUGCAUAUAAGCA	457	UGCUUAUAUGCAGCAUCUG	1195
UUGGGCCUGAAAAUCCAUA	458	UUGGGCCUGAAAAUCCAUA	458	UAUGGAUUUUCAGGCCCAA	1196
GCAUGGACAAGUAGACUGU	459	GCAUGGACAAGUAGACUGU	459	ACAGUCUACUUGUCCAUGC	1197
ACCUGUCAACAUAAUUGGA	460	ACCUGUCAACAUAAUUGGA	460	UCCAAUUAUGUUGACAGGU	1198
CAGGAACUACUAGUACCCU	461	CAGGAACUACUAGUACCCU	461	AGGGUACUAGUAGUUCCUG	1199
AUAGCAACAGACAUACAAA	462	AUAGCAACAGACAUACAAA	462	UUUGUAUGUCUGUUGCUAU	1200
GGAGAGAGAUGGGUGCGAG	463	GGAGAGAGAUGGGUGCGAG	463	CUCGCACCCAUCUCUCUCC	1201
ACACCUGUCAACAUAAUUG	464	ACACCUGUCAACAUAAUUG	464	CAAUUAUGUUGACAGGUGU	1202
AGAAAUGAUGACAGCAUGU	465	AGAAAUGAUGACAGCAUGU	465	ACAUGCUGUCAUCAUUUCU	1203
AGAAGGAGAGAGAUGGGUG	466	AGAAGGAGAGAGAUGGGUG	466	CACCCAUCUCUCUCCUUCU	1204
AAUCAUUCAAGCACAACCA	467	AAUCAUUCAAGCACAACCA	467	UGGUUGUGCUUGAAUGAUU	1205
CAAAAAUUGGGCCUGAAAA	468	CAAAAAUUGGGCCUGAAAA	468	UUUUCAGGCCCAAUUUUUG	1206
GCAGUACAAAUGGCAGUAU	469	GCAGUACAAAUGGCAGUAU	469	AUACUGCCAUUUGUACUGC	1207
GGGCAGUAGUAAUACAAGA	470	GGGCAGUAGUAAUACAAGA	470	UCUUGUAUUACUACUGCCC	1208
UCAUUCAAGCACAACCAGA	471	UCAUUCAAGCACAACCAGA	471	UCUGGUUGUGCUUGAAUGA	1209
AUGAUGACAGCAUGUCAGG	472	AUGAUGACAGCAUGUCAGG	472	CCUGACAUGCUGUCAUCAU	1210
GAACAAGUAGAUAAAUUAG	473	GAACAAGUAGAUAAAUUAG	473	CUAAUUUAUCUACUUGUUC	1211
UGACAGCAUGUCAGGGAGU	474	UGACAGCAUGUCAGGGAGU	474	ACUCCCUGACAUGCUGUCA	1212
GGAACUACUAGUACCCUUC	475	GGAACUACUAGUACCCUUC	475	GAAGGGUACUAGUAGUUCC	1213
CACCUGUCAACAUAAUUGG	476	CACCUGUCAACAUAAUUGG	476	CCAAUUAUGUUGACAGGUG	1214
GGCCAGAUGAGAGAACCAA	477	GGCCAGAUGAGAGAACCAA	477	UUGGUUCUCUCAUCUGGCC	1215
UGUGUACCCACAGACCCCA	478	UGUGUACCCACAGACCCCA	478	UGGGGUCUGUGGGUACACA	1216
GGAAUCAUUCAAGCACAAC	479	GGAAUCAUUCAAGCACAAC	479	GUUGUGCUUGAAUGAUUCC	1217
CAGUACAAAUGGCAGUAUU	480	CAGUACAAAUGGCAGUAUU	480	AAUACUGCCAUUUGUACUG	1218
GCAGGAAGAAGCGGAGACA	481	GCAGGAAGAAGCGGAGACA	481	UGUCUCCGCUUCUUCCUGC	1219
AAAGCCAGGAAUGGAUGGC	482	AAAGCCAGGAAUGGAUGGC	482	GCCAUCCAUUCCUGGCUUU	1220
UGAACAAGUAGAUAAAUUA	483	UGAACAAGUAGAUAAAUUA	483	UAAUUUAUCUACUUGUUCA	1221
CAAAAAUUCAAAAUUUUCG	484	CAAAAAUUCAAAAUUUUCG	484	CGAAAAUUUUGAAUUUUUG	1222
UAGGACCUACACCUGUCAA	485	UAGGACCUACACCUGUCAA	485	UUGACAGGUGUAGGUCCUA	1223
GCCAGAUGAGAGAACCAAG	486	GCCAGAUGAGAGAACCAAG	486	CUUGGUUCUCUCAUCUGGC	1224
GACAGCUGGACUGUCAAUG	487	GACAGCUGGACUGUCAAUG	487	CAUUGACAGUCCAGCUGUC	1225
AAAGCCACCUUUGCCUAGU	488	AAAGCCACCUUUGCCUAGU	488	ACUAGGCAAAGGUGGCUUU	1226
GAAAUGAACAAGUAGAUAA	489	GAAAUGAACAAGUAGAUAA	489	UUAUCUACUUGUUCAUUUC	1227
ACAAUUUUAAAAGAAAAGG	490	ACAAUUUUAAAAGAAAAGG	490	CCUUUUCUUUUAAAAUUGU	1228

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HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GCUGUGGAAAGAUACCUAA	491	GCUGUGGAAAGAUACCUAA	491	UUAGGUAUCUUUCCACAGC	1229
UGUCAACAUAAUUGGAAGA	492	UGUCAACAUAAUUGGAAGA	492	UCUUCCAAUUAUGUUGACA	1230
UAAAAGAAAAGGGGGGAUU	493	UAAAAGAAAAGGGGGGAUU	493	AAUCCCCCCUUUUCUUUUA	1231
CAAUUUUAAAAGAAAAGGG	494	CAAUUUUAAAAGAAAAGGG	494	CCCUUUUCUUUUAAAAUUG	1232
UUAGUAGAUUUCAGAGAAC	495	UUAGUAGAUUUCAGAGAAC	495	GUUCUCUGAAAUCUACUAA	1233
AAUUUUAAAAGAAAAGGGG	496	AAUUUUAAAAGAAAAGGGG	496	CCCCUUUUCUUUUAAAAUU	1234
UAGCAACAGACAUACAAAC	497	UAGCAACAGACAUACAAAC	497	GUUUGUAUGUCUGUUGCUA	1235
UGGAACAAGCCCCAGAAGA	498	UGGAACAAGCCCCAGAAGA	498	UCUUCUGGGGCUUGUUCCA	1236
AGGAUGAGGAUUAGAACAU	499	AGGAUGAGGAUUAGAACAU	499	AUGUUCUAAUCCUCAUCCU	1237
GACAAUUGGAGAAGUGAAU	500	GACAAUUGGAGAAGUGAAU	500	AUUCACUUCUCCAAUUGUC	1238
ACAGACCCCAACCCACAAG	501	ACAGACCCCAACCCACAAG	501	CUUGUGGGUUGGGGUCUGU	1239
CACCUAGAACUUUAAAUGC	502	CACCUAGAACUUUAAAUGC	502	GCAUUUAAAGUUCUAGGUG	1240
GAGCCAACAGCCCCACCAG	503	GAGCCAACAGCCCCACCAG	503	CUGGUGGGGCUGUUGGCUC	1241
AGGACCUACACCUGUCAAC	504	AGGACCUACACCUGUCAAC	504	GUUGACAGGUGUAGGUCCU	1242
UUACAAAAAUUCAAAAUUU	505	UUACAAAAAUUCAAAAUUU	505	AAAUUUUGAAUUUUUGUAA	1243
GGAGGUUUUAUCAAAGUAA	506	GGAGGUUUUAUCAAAGUAA	506	UUACUUUGAUAAAACCUCC	1244
CUGGCUGUGGAAAGAUACC	507	CUGGCUGUGGAAAGAUACC	507	GGUAUCUUUCCACAGCCAG	1245
GGAGAAGUGAAUUAUAUAA	508	GGAGAAGUGAAUUAUAUAA	508	UUAUAUAAUUCACUUCUCC	1246
AAUGAUGACAGCAUGUCAG	509	AAUGAUGACAGCAUGUCAG	509	CUGACAUGCUGUCAUCAUU	1247
AUCAUUAGGGAUUAUGGAA	510	AUCAUUAGGGAUUAUGGAA	510	UUCCAUAAUCCCUAAUGAU	1248
UCAAAAAUUGGGCCUGAAA	511	UCAAAAAUUGGGCCUGAAA	511	UUUCAGGCCCAAUUUUUGA	1249
ACCUACACCUGUCAACAUA	512	ACCUACACCUGUCAACAUA	512	UAUGUUGACAGGUGUAGGU	1250
GAUGAGGAUUAGAACAUGG	513	GAUGAGGAUUAGAACAUGG	513	CCAUGUUCUAAUCCUCAUC	1251
ACAGCUGGACUGUCAAUGA	514	ACAGCUGGACUGUCAAUGA	514	UCAUUGACAGUCCAGCUGU	1252
CCCUCAGAUGCUGCAUAUA	515	CCCUCAGAUGCUGCAUAUA	515	UAUAUGCAGCAUCUGAGGG	1253
AUUAGUAGAUUUCAGAGAA	516	AUUAGUAGAUUUCAGAGAA	516	UUCUCUGAAAUCUACUAAU	1254
AGAAAGAGCAGAAGACAGU	517	AGAAAGAGCAGAAGACAGU	517	ACUGUCUUCUGCUCUUUCU	1255
GACCUACACCUGUCAACAU	518	GACCUACACCUGUCAACAU	518	AUGUUGACAGGUGUAGGUC	1256
CACUCUUUGGCAACGACCC	519	CACUCUUUGGCAACGACCC	519	GGGUCGUUGCCAAAGAGUG	1257
AUGAGGAUUAGAACAUGGA	520	AUGAGGAUUAGAACAUGGA	520	UCCAUGUUCUAAUCCUCAU	1258
AUUUUAAAAGAAAAGGGGG	521	AUUUUAAAAGAAAAGGGGG	521	CCCCCUUUUCUUUUAAAAU	1259
AGAACUUUAAAUGCAUGGG	522	AGAACUUUAAAUGCAUGGG	522	CCCAUGCAUUUAAAGUUCU	1260
AUCUAUCAAUACAUGGAUG	523	AUCUAUCAAUACAUGGAUG	523	CAUCCAUGUAUUGAUAGAU	1261
AUGGAACAAGCCCCAGAAG	524	AUGGAACAAGCCCCAGAAG	524	CUUCUGGGGCUUGUUCCAU	1262
UUAUGACCCAUCAAAAGAC	525	UUAUGACCCAUCAAAAGAC	525	GUCUUUUGAUGGGUCAUAA	1263
CACAAUUUUAAAAGAAAAG	526	CACAAUUUUAAAAGAAAAG	526	CUUUUCUUUUAAAAUUGUG	1264

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HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GAACUUUAAAUGCAUGGGU	527	GAACUUUAAAUGCAUGGGU	527	ACCCAUGCAUUUAAAGUUC	1265
AAAAGAAAAGGGGGGAUUG	528	AAAAGAAAAGGGGGGAUUG	528	CAAUCCCCCCUUUUCUUUU	1266
GGAUGGAAAGGAUCACCAG	529	GGAUGGAAAGGAUCACCAG	529	CUGGUGAUCCUUUCCAUCC	1267
AGGGGCAGUAGUAAUACAA	530	AGGGGCAGUAGUAAUACAA	530	UUGUAUUACUACUGCCCCU	1268
AAAGGGGGGAUUGGGGGGU	531	AAAGGGGGGAUUGGGGGGU	531	ACCCCCCAAUCCCCCCUUU	1269
AAGGGGGGAUUGGGGGGUA	532	AAGGGGGGAUUGGGGGGUA	532	UACCCCCCAAUCCCCCCUU	1270
CAGGAUGAGGAUUAGAACA	533	CAGGAUGAGGAUUAGAACA	533	UGUUCUAAUCCUCAUCCUG	1271
AAAAUUAGUAGAUUUCAGA	534	AAAAUUAGUAGAUUUCAGA	534	UCUGAAAUCUACUAAUUUU	1272
GAAUUGGAGGAAAUGAACA	535	GAAUUGGAGGAAAUGAACA	535	UGUUCAUUUCCUCCAAUUC	1273
UACAAAAAUUCAAAAUUUU	536	UACAAAAAUUCAAAAUUUU	536	AAAAUUUUGAAUUUUUGUA	1274
AGGAACUACUAGUACCCUU	537	AGGAACUACUAGUACCCUU	537	AAGGGUACUAGUAGUUCCU	1275
AAAGAAAAGGGGGGAUUGG	538	AAAGAAAAGGGGGGAUUGG	538	CCAAUCCCCCCUUUUCUUU	1276
AAAAAUUGGAUGACAGAAA	539	AAAAAUUGGAUGACAGAAA	539	UUUCUGUCAUCCAAUUUUU	1277
ACAGGAUGAGGAUUAGAAC	540	ACAGGAUGAGGAUUAGAAC	540	GUUCUAAUCCUCAUCCUGU	1278
ACAAUUGGAGAAGUGAAUU	541	ACAAUUGGAGAAGUGAAUU	541	AAUUCACUUCUCCAAUUGU	1279
GGAUGAGGAUUAGAACAUG	542	GGAUGAGGAUUAGAACAUG	542	CAUGUUCUAAUCCUCAUCC	1280
UCACCUAGAACUUUAAAUG	543	UCACCUAGAACUUUAAAUG	543	CAUUUAAAGUUCUAGGUGA	1281
AUUGGGCCUGAAAAUCCAU	544	AUUGGGCCUGAAAAUCCAU	544	AUGGAUUUUCAGGCCCAAU	1282
AAUUGGGCCUGAAAAUCCA	545	AAUUGGGCCUGAAAAUCCA	545	UGGAUUUUCAGGCCCAAUU	1283
GGACCUACACCUGUCAACA	546	GGACCUACACCUGUCAACA	546	UGUUGACAGGUGUAGGUCC	1284
GACAGGAUGAGGAUUAGAA	547	GACAGGAUGAGGAUUAGAA	547	UUCUAAUCCUCAUCCUGUC	1285
UCUAUCAAUACAUGGAUGA	548	UCUAUCAAUACAUGGAUGA	548	UCAUCCAUGUAUUGAUAGA	1286
GGAAUUGGAGGAAAUGAAC	549	GGAAUUGGAGGAAAUGAAC	549	GUUCAUUUCCUCCAAUUCC	1287
AAAAGGGGGGAUUGGGGGG	550	AAAAGGGGGGAUUGGGGGG	550	CCCCCCAAUCCCCCCUUUU	1288
AAAAUUGGAUGACAGAAAC	551	AAAAUUGGAUGACAGAAAC	551	GUUUCUGUCAUCCAAUUUU	1289
CAAUUGGAGAAGUGAAUUA	552	CAAUUGGAGAAGUGAAUUA	552	UAAUUCACUUCUCCAAUUG	1290
AUGACCCAUCAAAAGACUU	553	AUGACCCAUCAAAAGACUU	553	AAGUCUUUUGAUGGGUCAU	1291
CUUAAGCCUCAAUAAAGCU	554	CUUAAGCCUCAAUAAAGCU	554	AGCUUUAUUGAGGCUUAAG	1292
AGUACAAUGUGCUUCCACA	555	AGUACAAUGUGCUUCCACA	555	UGUGGAAGCACAUUGUACU	1293
UUUCCGCUGGGGACUUUCC	556	UUUCCGCUGGGGACUUUCC	556	GGAAAGUCCCCAGCGGAAA	1294
CAGACAUACAAACUAAAGA	557	CAGACAUACAAACUAAAGA	557	UCUUUAGUUUGUAUGUCUG	1295
UUAAGCCUCAAUAAAGCUU	558	UUAAGCCUCAAUAAAGCUU	558	AAGCUUUAUUGAGGCUUAA	1296
GGACAAUUGGAGAAGUGAA	559	GGACAAUUGGAGAAGUGAA	559	UUCACUUCUCCAAUUGUCC	1297
GGAUUGGGGGGUACAGUGC	560	GGAUUGGGGGGUACAGUGC	560	GCACUGUACCCCCCAAUCC	1298
AAAUUGGGCCUGAAAAUCC	561	AAAUUGGGCCUGAAAAUCC	561	GGAUUUUCAGGCCCAAUUU	1299

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
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GUGGGGGGACAUCAAGCAG	563	GUGGGGGGACAUCAAGCAG	563	CUGCUUGAUGUCCCCCCAC	1301
UCCUGGCUGUGGAAAGAUA	564	UCCUGGCUGUGGAAAGAUA	564	UAUCUUUCCACAGCCAGGA	1302
ACAAAAAUUCAAAAUUUUC	565	ACAAAAAUUCAAAAUUUUC	565	GAAAAUUUUGAAUUUUUGU	1303
GGGGAUUGGGGGGUACAGU	566	GGGGAUUGGGGGGUACAGU	566	ACUGUACCCCCCAAUCCCC	1304
UAAACACAGUGGGGGGACA	567	UAAACACAGUGGGGGGACA	567	UGUCCCCCCACUGUGUUUA	1305
CAGACCCCAACCCACAAGA	568	CAGACCCCAACCCACAAGA	568	UCUUGUGGGUUGGGGUCUG	1306
AGGGGCAAAUGGUACAUCA	569	AGGGGCAAAUGGUACAUCA	569	UGAUGUACCAUUUGCCCCU	1307
AAUUGGAGGAAAUGAACAA	570	AAUUGGAGGAAAUGAACAA	570	UUGUUCAUUUCCUCCAAUU	1308
AAGCCACCUUUGCCUAGUG	571	AAGCCACCUUUGCCUAGUG	571	CACUAGGCAAAGGUGGCUU	1309
CCAUGUUUUCAGCAUUAUC	572	CCAUGUUUUCAGCAUUAUC	572	GAUAAUGCUGAAAACAUGG	1310
AAAGAAAAAAUCAGUAACA	573	AAAGAAAAAAUCAGUAACA	573	UGUUACUGAUUUUUUCUUU	1311
AAAAAAUUGGAUGACAGAA	574	AAAAAAUUGGAUGACAGAA	574	UUCUGUCAUCCAAUUUUUU	1312
CAGUACAAUGUGCUUCCAC	575	CAGUACAAUGUGCUUCCAC	575	GUGGAAGCACAUUGUACUG	1313
CUUUCCGCUGGGGACUUUC	576	CUUUCCGCUGGGGACUUUC	576	GAAAGUCCCCAGCGGAAAG	1314
GCAACAGACAUACAAACUA	577	GCAACAGACAUACAAACUA	577	UAGUUUGUAUGUCUGUUGC	1315
UAUCACCUAGAACUUUAAA	578	UAUCACCUAGAACUUUAAA	578	UUUAAAGUUCUAGGUGAUA	1316
ACCCACAGACCCCAACCCA	579	ACCCACAGACCCCAACCCA	579	UGGGUUGGGGUCUGUGGGU	1317
GAUAGAUGGAACAAGCCCC	580	GAUAGAUGGAACAAGCCCC	580	GGGGCUUGUUCCAUCUAUC	1318
GCUUAAGCCUCAAUAAAGC	581	GCUUAAGCCUCAAUAAAGC	581	GCUUUAUUGAGGCUUAAGC	1319
AUUGGGGGGUACAGUGCAG	582	AUUGGGGGGUACAGUGCAG	582	CUGCACUGUACCCCCCAAU	1320
CCCACAGACCCCAACCCAC	583	CCCACAGACCCCAACCCAC	583	GUGGGUUGGGGUCUGUGGG	1321
AAAAUUGGGCCUGAAAAUC	584	AAAAUUGGGCCUGAAAAUC	584	GAUUUUCAGGCCCAAUUUU	1322
CAUUCAAGCACAACCAGAU	585	CAUUCAAGCACAACCAGAU	585	AUCUGGUUGUGCUUGAAUG	1323
ACUUUAAAUGCAUGGGUAA	586	ACUUUAAAUGCAUGGGUAA	586	UUACCCAUGCAUUUAAAGU	1324
UAGAACUUUAAAUGCAUGG	587	UAGAACUUUAAAUGCAUGG	587	CCAUGCAUUUAAAGUUCUA	1325
CUUUAAAUGCAUGGGUAAA	588	CUUUAAAUGCAUGGGUAAA	588	UUUACCCAUGCAUUUAAAG	1326
GGGAUUGGGGGGUACAGUG	589	GGGAUUGGGGGGUACAGUG	589	CACUGUACCCCCCAAUCCC	1327
UAUGACCCAUCAAAAGACU	590	UAUGACCCAUCAAAAGACU	590	AGUCUUUUGAUGGGUCAUA	1328
GAAGAAGCGGAGACAGCGA	591	GAAGAAGCGGAGACAGCGA	591	UCGCUGUCUCCGCUUCUUC	1329
CCCAUGUUUUCAGCAUUAU	592	CCCAUGUUUUCAGCAUUAU	592	AUAAUGCUGAAAACAUGGG	1330
AGGAAUUGGAGGAAAUGAA	593	AGGAAUUGGAGGAAAUGAA	593	UUCAUUUCCUCCAAUUCCU	1331
AGAGACAGGCUAAUUUUUU	594	AGAGACAGGCUAAUUUUUU	594	AAAAAAUUAGCCUGUCUCU	1332
AAGUAGAUAAAUUAGUCAG	595	AAGUAGAUAAAUUAGUCAG	595	CUGACUAAUUUAUCUACUU	1333
AUGUUUUCAGCAUUAUCAG	596	AUGUUUUCAGCAUUAUCAG	596	CUGAUAAUGCUGAAAACAU	1334
UUAUUGUCUGGUAUAGUGC	597	UUAUUGUCUGGUAUAGUGC	597	GCACUAUACCAGACAAUAA	1335

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AUUACAAAAAUUCAAAAUU	598	AUUACAAAAAUUCAAAAUU	598	AAUUUUGAAUUUUUGUAAU	1336
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CCUGGCUGUGGAAAGAUAC	600	CCUGGCUGUGGAAAGAUAC	600	GUAUCUUUCCACAGCCAGG	1338
UGUUUUCAGCAUUAUCAGA	601	UGUUUUCAGCAUUAUCAGA	601	UCUGAUAAUGCUGAAAACA	1339
ACCUAGAACUUUAAAUGCA	602	ACCUAGAACUUUAAAUGCA	602	UGCAUUUAAAGUUCUAGGU	1340
GGGAUGGAAAGGAUCACCA	603	GGGAUGGAAAGGAUCACCA	603	UGGUGAUCCUUUCCAUCCC	1341
AAUUAAAGCCAGGAAUGGA	604	AAUUAAAGCCAGGAAUGGA	604	UCCAUUCCUGGCUUUAAUU	1342
AAAGGAAUUGGAGGAAAUG	605	AAAGGAAUUGGAGGAAAUG	605	CAUUUCCUCCAAUUCCUUU	1343
ACUUUCCGCUGGGGACUUU	606	ACUUUCCGCUGGGGACUUU	606	AAAGUCCCCAGCGGAAAGU	1344
ACAGAAGAAAAAAUAAAAG	607	ACAGAAGAAAAAAUAAAAG	607	CUUUUAUUUUUUCUUCUGU	1345
AGCAACAGACAUACAAACU	608	AGCAACAGACAUACAAACU	608	AGUUUGUAUGUCUGUUGCU	1346
UAUUGUCUGGUAUAGUGCA	609	UAUUGUCUGGUAUAGUGCA	609	UGCACUAUACCAGACAAUA	1347
UUAAAAGAAAAGGGGGGAU	610	UUAAAAGAAAAGGGGGGAU	610	AUCCCCCCUUUUCUUUUAA	1348
UGCUUAAGCCUCAAUAAAG	611	UGCUUAAGCCUCAAUAAAG	611	CUUUAUUGAGGCUUAAGCA	1349
CAGGAAGAUGGCCAGUAAA	612	CAGGAAGAUGGCCAGUAAA	612	UUUACUGGCCAUCUUCCUG	1350
CCAGAUGAGAGAACCAAGG	613	CCAGAUGAGAGAACCAAGG	613	CCUUGGUUCUCUCAUCUGG	1351
GAUUGGGGGGUACAGUGCA	614	GAUUGGGGGGUACAGUGCA	614	UGCACUGUACCCCCCAAUC	1352
AAAUGAACAAGUAGAUAAA	615	AAAUGAACAAGUAGAUAAA	615	UUUAUCUACUUGUUCAUUU	1353
AGCCACCUUUGCCUAGUGU	616	AGCCACCUUUGCCUAGUGU	616	ACACUAGGCAAAGGUGGCU	1354
GACUUUCCGCUGGGGACUU	617	GACUUUCCGCUGGGGACUU	617	AAGUCCCCAGCGGAAAGUC	1355
CCAGUAAAAUUAAAGCCAG	618	CCAGUAAAAUUAAAGCCAG	618	CUGGCUUUAAUUUUACUGG	1356
GCAAUGUAUGCCCCUCCCA	619	GCAAUGUAUGCCCCUCCCA	619	UGGGAGGGGCAUACAUUGC	1357
AACUUUAAAUGCAUGGGUA	620	AACUUUAAAUGCAUGGGUA	620	UACCCAUGCAUUUAAAGUU	1358
UUGGGGGGUACAGUGCAGG	621	UUGGGGGGUACAGUGCAGG	621	CCUGCACUGUACCCCCCAA	1359
GGACUUUCCGCUGGGGACU	622	GGACUUUCCGCUGGGGACU	622	AGUCCCCAGCGGAAAGUCC	1360
CUAGAACUUUAAAUGCAUG	623	CUAGAACUUUAAAUGCAUG	623	CAUGCAUUUAAAGUUCUAG	1361
UCAGUACAAUGUGCUUCCA	624	UCAGUACAAUGUGCUUCCA	624	UGGAAGCACAUUGUACUGA	1362
AAGGAAUUGGAGGAAAUGA	625	AAGGAAUUGGAGGAAAUGA	625	UCAUUUCCUCCAAUUCCUU	1363
UACCCACAGACCCCAACCC	626	UACCCACAGACCCCAACCC	626	GGGUUGGGGUCUGUGGGUA	1364
GAGACAGGCUAAUUUUUUA	627	GAGACAGGCUAAUUUUUUA	627	UAAAAAAUUAGCCUGUCUC	1365
CUGCUUAAGCCUCAAUAAA	628	CUGCUUAAGCCUCAAUAAA	628	UUUAUUGAGGCUUAAGCAG	1366
AGGAAGAUGGCCAGUAAAA	629	AGGAAGAUGGCCAGUAAAA	629	UUUUACUGGCCAUCUUCCU	1367
AGACAUACAAACUAAAGAA	630	AGACAUACAAACUAAAGAA	630	UUCUUUAGUUUGUAUGUCU	1368
CAUGUUUUCAGCAUUAUCA	631	CAUGUUUUCAGCAUUAUCA	631	UGAUAAUGCUGAAAACAUG	1369
UUGGAAAGGACCAGCAAAG	632	UUGGAAAGGACCAGCAAAG	632	CUUUGCUGGUCCUUUCCAA	1370

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
GGCUGUUGGAAAUGUGGAA	633	GGCUGUUGGAAAUGUGGAA	633	UUCCACAUUUCCAACAGCC	1371
UAAAUGGAGAAAAUUAGUA	634	UAAAUGGAGAAAAUUAGUA	634	UACUAAUUUUCUCCAUUUA	1372
AGGAAGAAGCGGAGACAGC	635	AGGAAGAAGCGGAGACAGC	635	GCUGUCUCCGCUUCUUCCU	1373
AAAAAAGAAAAAAUCAGUA	636	AAAAAAGAAAAAAUCAGUA	636	UACUGAUUUUUUCUUUUUU	1374
AUCAGAAAGAACCUCCAUU	637	AUCAGAAAGAACCUCCAUU	637	AAUGGAGGUUCUUUCUGAU	1375
AGACCCCAACCCACAAGAA	638	AGACCCCAACCCACAAGAA	638	UUCUUGUGGGUUGGGGUCU	1376
CAAGUAGAUAAAUUAGUCA	639	CAAGUAGAUAAAUUAGUCA	639	UGACUAAUUUAUCUACUUG	1377
AAAGCUAUAGGUACAGUAU	640	AAAGCUAUAGGUACAGUAU	640	AUACUGUACCUAUAGCUUU	1378
UGCUGCAUAUAAGCAGCUG	641	UGCUGCAUAUAAGCAGCUG	641	CAGCUGCUUAUAUGCAGCA	1379
UUUAAAUGCAUGGGUAAAA	642	UUUAAAUGCAUGGGUAAAA	642	UUUUACCCAUGCAUUUAAA	1380
UUUUCAGCAUUAUCAGAAG	643	UUUUCAGCAUUAUCAGAAG	643	CUUCUGAUAAUGCUGAAAA	1381
ACUGCUUAAGCCUCAAUAA	644	ACUGCUUAAGCCUCAAUAA	644	UUAUUGAGGCUUAAGCAGU	1382
GGAAAGGACCAGCAAAGCU	645	GGAAAGGACCAGCAAAGCU	645	AGCUUUGCUGGUCCUUUCC	1383
UGUACCAGUAAAAUUAAAG	646	UGUACCAGUAAAAUUAAAG	646	CUUUAAUUUUACUGGUACA	1384
GAAGAAAAAAUAAAAGCAU	647	GAAGAAAAAAUAAAAGCAU	647	AUGCUUUUAUUUUUUCUUC	1385
GUGUACCCACAGACCCCAA	648	GUGUACCCACAGACCCCAA	648	UUGGGGUCUGUGGGUACAC	1386
GGGGGGAUUGGGGGGUACA	649	GGGGGGAUUGGGGGGUACA	649	UGUACCCCCCAAUCCCCCC	1387
GGAAGAAGCGGAGACAGCG	650	GGAAGAAGCGGAGACAGCG	650	CGCUGUCUCCGCUUCUUCC	1388
GAAGCGGAGACAGCGACGA	651	GAAGCGGAGACAGCGACGA	651	UCGUCGCUGUCUCCGCUUC	1389
UUAAAUGCAUGGGUAAAAG	652	UUAAAUGCAUGGGUAAAAG	652	CUUUUACCCAUGCAUUUAA	1390
AACCCACUGCUUAAGCCUC	653	AACCCACUGCUUAAGCCUC	653	GAGGCUUAAGCAGUGGGUU	1391
GUUUUCAGCAUUAUCAGAA	654	GUUUUCAGCAUUAUCAGAA	654	UUCUGAUAAUGCUGAAAAC	1392
GGAUUAAAUAAAAUAGUAA	655	GGAUUAAAUAAAAUAGUAA	655	UUACUAUUUUAUUUAAUCC	1393
GUACCCACAGACCCCAACC	656	GUACCCACAGACCCCAACC	656	GGUUGGGGUCUGUGGGUAC	1394
GAUUAAAUAAAAUAGUAAG	657	GAUUAAAUAAAAUAGUAAG	657	CUUACUAUUUUAUUUAAUC	1395
AAGCCUCAAUAAAGCUUGC	658	AAGCCUCAAUAAAGCUUGC	658	GCAAGCUUUAUUGAGGCUU	1396
GCAGGACAUAACAAGGUAG	659	GCAGGACAUAACAAGGUAG	659	CUACCUUGUUAUGUCCUGC	1397
CCCACUGCUUAAGCCUCAA	660	CCCACUGCUUAAGCCUCAA	660	UUGAGGCUUAAGCAGUGGG	1398
GGGACUUUCCGCUGGGGAC	661	GGGACUUUCCGCUGGGGAC	661	GUCCCCAGCGGAAAGUCCC	1399
AUCACCUAGAACUUUAAAU	662	AUCACCUAGAACUUUAAAU	662	AUUUAAAGUUCUAGGUGAU	1400
UAGAGCCCUGGAAGCAUCC	663	UAGAGCCCUGGAAGCAUCC	663	GGAUGCUUCCAGGGCUCUA	1401
GGGCUGUUGGAAAUGUGGA	664	GGGCUGUUGGAAAUGUGGA	664	UCCACAUUUCCAACAGCCC	1402
UUUCAGCAUUAUCAGAAGG	665	UUUCAGCAUUAUCAGAAGG	665	CCUUCUGAUAAUGCUGAAA	1403
UGACCCAUCAAAAGACUUA	666	UGACCCAUCAAAAGACUUA	666	UAAGUCUUUUGAUGGGUCA	1404
AGAAAAAAUAAAAGCAUUA	667	AGAAAAAAUAAAAGCAUUA	667	UAAUGCUUUUAUUUUUUCU	1405
AGAAGCGGAGACAGCGACG	668	AGAAGCGGAGACAGCGACG	668	CGUCGCUGUCUCCGCUUCU	1406

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AAGAAAAAAUAAAAGCAUU	669	AAGAAAAAAUAAAAGCAUU	669	AAUGCUUUUAUUUUUUCUU	1407
AAUGGAGAAAAUUAGUAGA	670	AAUGGAGAAAAUUAGUAGA	670	UCUACUAAUUUUCUCCAUU	1408
GCUGAACAUCUUAAGACAG	671	GCUGAACAUCUUAAGACAG	671	CUGUCUUAAGAUGUUCAGC	1409
AAAAAGAAAAAAUCAGUAA	672	AAAAAGAAAAAAUCAGUAA	672	UUACUGAUUUUUUCUUUUU	1410
GAACAAGCCCCAGAAGACC	673	GAACAAGCCCCAGAAGACC	673	GGUCUUCUGGGGCUUGUUC	1411
GUGAUAAAUGUCAGCUAAA	674	GUGAUAAAUGUCAGCUAAA	674	UUUAGCUGACAUUUAUCAC	1412
GAGCCCUGGAAGCAUCCAG	675	GAGCCCUGGAAGCAUCCAG	675	CUGGAUGCUUCCAGGGCUC	1413
AGUGGGGGGACAUCAAGCA	676	AGUGGGGGGACAUCAAGCA	676	UGCUUGAUGUCCCCCCACU	1414
GCCUGGGAGCUCUCUGGCU	677	GCCUGGGAGCUCUCUGGCU	677	AGCCAGAGAGCUCCCAGGC	1415
UGGAAAGGACCAGCAAAGC	678	UGGAAAGGACCAGCAAAGC	678	GCUUUGCUGGUCCUUUCCA	1416
AGCAGGACAUAACAAGGUA	679	AGCAGGACAUAACAAGGUA	679	UACCUUGUUAUGUCCUGCU	1417
CCUAGAACUUUAAAUGCAU	680	CCUAGAACUUUAAAUGCAU	680	AUGCAUUUAAAGUUCUAGG	1418
AGUAGAUAAAUUAGUCAGU	681	AGUAGAUAAAUUAGUCAGU	681	ACUGACUAAUUUAUCUACU	1419
AAAUUAAAGCCAGGAAUGG	682	AAAUUAAAGCCAGGAAUGG	682	CCAUUCCUGGCUUUAAUUU	1420
AGUAAAAUUAAAGCCAGGA	683	AGUAAAAUUAAAGCCAGGA	683	UCCUGGCUUUAAUUUUACU	1421
UGUGAUAAAUGUCAGCUAA	684	UGUGAUAAAUGUCAGCUAA	684	UUAGCUGACAUUUAUCACA	1422
AGCCCUGGAAGCAUCCAGG	685	AGCCCUGGAAGCAUCCAGG	685	CCUGGAUGCUUCCAGGGCU	1423
CACUGCUUAAGCCUCAAUA	686	CACUGCUUAAGCCUCAAUA	686	UAUUGAGGCUUAAGCAGUG	1424
AAAAAAUCAGUAACAGUAC	687	AAAAAAUCAGUAACAGUAC	687	GUACUGUUACUGAUUUUUU	1425
GAGCCUGGGAGCUCUCUGG	688	GAGCCUGGGAGCUCUCUGG	688	CCAGAGAGCUCCCAGGCUC	1426
UUCCGCUGGGGACUUUCCA	689	UUCCGCUGGGGACUUUCCA	689	UGGAAAGUCCCCAGCGGAA	1427
GAGAGACAGGCUAAUUUUU	690	GAGAGACAGGCUAAUUUUU	690	AAAAAUUAGCCUGUCUCUC	1428
GCUGUGAUAAAUGUCAGCU	691	GCUGUGAUAAAUGUCAGCU	691	AGCUGACAUUUAUCACAGC	1429
CCACAGACCCCAACCCACA	692	CCACAGACCCCAACCCACA	692	UGUGGGUUGGGGUCUGUGG	1430
CAGGAAGAAGCGGAGACAG	693	CAGGAAGAAGCGGAGACAG	693	CUGUCUCCGCUUCUUCCUG	1431
UAAGCCUCAAUAAAGCUUG	694	UAAGCCUCAAUAAAGCUUG	694	CAAGCUUUAUUGAGGCUUA	1432
UAAAAAAGAAAAAAUCAGU	695	UAAAAAAGAAAAAAUCAGU	695	ACUGAUUUUUUCUUUUUUA	1433
GACAGAAGAAAAAAUAAAA	696	GACAGAAGAAAAAAUAAAA	696	UUUUAUUUUUUCUUCUGUC	1434
GUACCAGUAAAAUUAAAGC	697	GUACCAGUAAAAUUAAAGC	697	GCUUUAAUUUUACUGGUAC	1435
AAAAGAAAAAAUCAGUAAC	698	AAAAGAAAAAAUCAGUAAC	698	GUUACUGAUUUUUUCUUUU	1436
AAAAAUCAGUAACAGUACU	699	AAAAAUCAGUAACAGUACU	699	AGUACUGUUACUGAUUUUU	1437
AGAGCCCUGGAAGCAUCCA	700	AGAGCCCUGGAAGCAUCCA	700	UGGAUGCUUCCAGGGCUCU	1438
CAGGGGCAAAUGGUACAUC	701	CAGGGGCAAAUGGUACAUC	701	GAUGUACCAUUUGCCCCUG	1439
CUGCAUUUACCAUACCUAG	702	CUGCAUUUACCAUACCUAG	702	CUAGGUAUGGUAAAUGCAG	1440
UAAAUGCAUGGGUAAAAGU	703	UAAAUGCAUGGGUAAAAGU	703	ACUUUUACCCAUGCAUUUA	1441

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
AAGUAAACAUAGUAACAGA	704	AAGUAAACAUAGUAACAGA	704	UCUGUUACUAUGUUUACUU	1442
CCACACAUGCCUGUGUACC	705	CCACACAUGCCUGUGUACC	705	GGUACACAGGCAUGUGUGG	1443
AGUAGAUUUCAGAGAACUU	706	AGUAGAUUUCAGAGAACUU	706	AAGUUCUCUGAAAUCUACU	1444
CAUCAGAAAGAACCUCCAU	707	CAUCAGAAAGAACCUCCAU	707	AUGGAGGUUCUUUCUGAUG	1445
ACCAGUAAAAUUAAAGCCA	708	ACCAGUAAAAUUAAAGCCA	708	UGGCUUUAAUUUUACUGGU	1446
CACAGACCCCAACCCACAA	709	CACAGACCCCAACCCACAA	709	UUGUGGGUUGGGGUCUGUG	1447
AGGGGGGAUUGGGGGGUAC	710	AGGGGGGAUUGGGGGGUAC	710	GUACCCCCCAAUCCCCCCU	1448
UGCAUUUACCAUACCUAGU	711	UGCAUUUACCAUACCUAGU	711	ACUAGGUAUGGUAAAUGCA	1449
CAAUGGACAUAUCAAAUUU	712	CAAUGGACAUAUCAAAUUU	712	AAAUUUGAUAUGUCCAUUG	1450
CUGAACAUCUUAAGACAGC	713	CUGAACAUCUUAAGACAGC	713	GCUGUCUUAAGAUGUUCAG	1451
GCCUCAAUAAAGCUUGCCU	714	GCCUCAAUAAAGCUUGCCU	714	AGGCAAGCUUUAUUGAGGC	1452
UGUACCCACAGACCCCAAC	715	UGUACCCACAGACCCCAAC	715	GUUGGGGUCUGUGGGUACA	1453
GAAGUAAACAUAGUAACAG	716	GAAGUAAACAUAGUAACAG	716	CUGUUACUAUGUUUACUUC	1454
GUAGGACCUACACCUGUCA	717	GUAGGACCUACACCUGUCA	717	UGACAGGUGUAGGUCCUAC	1455
CAGUGGGGGGACAUCAAGC	718	CAGUGGGGGGACAUCAAGC	718	GCUUGAUGUCCCCCCACUG	1456
ACCCACUGCUUAAGCCUCA	719	ACCCACUGCUUAAGCCUCA	719	UGAGGCUUAAGCAGUGGGU	1457
AAAAAUUGGGCCUGAAAAU	720	AAAAAUUGGGCCUGAAAAU	720	AUUUUCAGGCCCAAUUUUU	1458
UGGGGGGACAUCAAGCAGC	721	UGGGGGGACAUCAAGCAGC	721	GCUGCUUGAUGUCCCCCCA	1459
GUACAAAUGGCAGUAUUCA	722	GUACAAAUGGCAGUAUUCA	722	UGAAUACUGCCAUUUGUAC	1460
AAGCUAUAGGUACAGUAUU	723	AAGCUAUAGGUACAGUAUU	723	AAUACUGUACCUAUAGCUU	1461
CAGAAGAAAAAAUAAAAGC	724	CAGAAGAAAAAAUAAAAGC	724	GCUUUUAUUUUUUCUUCUG	1462
AAAUGCAUGGGUAAAAGUA	725	AAAUGCAUGGGUAAAAGUA	725	UACUUUUACCCAUGCAUUU	1463
AGCCUCAAUAAAGCUUGCC	726	AGCCUCAAUAAAGCUUGCC	726	GGCAAGCUUUAUUGAGGCU	1464
CCACUGCUUAAGCCUCAAU	727	CCACUGCUUAAGCCUCAAU	727	AUUGAGGCUUAAGCAGUGG	1465
AAGAAGCGGAGACAGCGAC	728	AAGAAGCGGAGACAGCGAC	728	GUCGCUGUCUCCGCUUCUU	1466
AAAUGGAGAAAAUUAGUAG	729	AAAUGGAGAAAAUUAGUAG	729	CUACUAAUUUUCUCCAUUU	1467
AGCCUGGGAGCUCUCUGGC	730	AGCCUGGGAGCUCUCUGGC	730	GCCAGAGAGCUCCCAGGCU	1468
AACAAGCCCCAGAAGACCA	731	AACAAGCCCCAGAAGACCA	731	UGGUCUUCUGGGGCUUGUU	1469
UACCAGUAAAAUUAAAGCC	732	UACCAGUAAAAUUAAAGCC	732	GGCUUUAAUUUUACUGGUA	1470
UUCAAAAAUUGGGCCUGAA	733	UUCAAAAAUUGGGCCUGAA	733	UUCAGGCCCAAUUUUUGAA	1471
AGAAGAAAAAAUAAAAGCA	734	AGAAGAAAAAAUAAAAGCA	734	UGCUUUUAUUUUUUCUUCU	1472
CUGUGUACCCACAGACCCC	735	CUGUGUACCCACAGACCCC	735	GGGGUCUGUGGGUACACAG	1473
GCCUGUACUGGGUCUCUCU	736	GCCUGUACUGGGUCUCUCU	736	AGAGAGACCCAGUACAGGC	1474

TABLE I-continued

HIV target and siRNA sequences

Sequence	Seq ID	Upper seq	Seq ID	Lower seq	Seq ID
CAGUAAAAUUAAAGCCAGG	737	CAGUAAAAUUAAAGCCAGG	737	CCUGGCUUUAAUUUUACUG	1475
UACAAAUGGCAGUAUUCAU	738	UACAAAUGGCAGUAUUCAU	738	AUGAAUACUGCCAUUUGUA	1476

HIV = NM_000633
The 3'-ends of the Upper sequence and the Lower sequence of the siRNA construct can include a overhang sequence, for example 1, 2, 3, or 4 nucleotides in length, preferably 2 nucleotides in length, wherein the overhanging sequence of the lower sequence is optionally complimentary to a portion of the target sequence. The upper sequence is also referred to as the sense strand, whereas the lower sequence is also referred to as the antisense strand.

[0262]

TABLE II

A. 2.5 μmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* RNA
Phosphoramidites	6.5	163 μL	45 sec	2.5 min	7.5 min
S-Ethyl Tetrazole	23.8	238 μL	45 sec	2.5 min	7.5 min
Acetic Anhydride	100	233 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	186	233 μL	5 sec	5 sec	5 sec
TCA	176	2.3 mL	21 sec	21 sec	21 sec
Iodine	11.2	1.7 mL	45 sec	45 sec	45 sec
Beaucage	12.9	645 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	6.67 mL	NA	NA	NA

B. 0.2 μmol Synthesis Cycle ABI 394 Instrument

Reagent	Equivalents	Amount	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* RNA
Phosphoramidites	15	31 μL	45 sec	233 sec	465 sec
S-Ethyl Tetrazole	38.7	31 μL	45 sec	233 mm	465 sec
Acetic Anhydride	655	124 μL	5 sec	5 sec	5 sec
N-Methyl Imidazole	1245	124 μL	5 sec	5 sec	5 sec
TCA	700	732 μL	10 sec	10 sec	10 sec
Iodine	20.6	244 μL	15 sec	15 sec	15 sec
Beaucage	7.7	232 μL	100 sec	300 sec	300 sec
Acetonitrile	NA	2.64 mL	NA	NA	NA

C. 0.2 μmol Synthesis Cycle 96 well Instrument

Reagent	Equivalents: DNA/ 2'-O-methyl/Ribo	Amount: DNA/2'-O-methyl/Ribo	Wait Time* DNA	Wait Time* 2'-O-methyl	Wait Time* Ribo
Phosphoramidites	22/33/66	40/60/120 μL	60 sec	180 sec	36O sec
S-Ethyl Tetrazole	70/105/210	40/60/120 μL	60 sec	180 min	360 sec
Acetic Anhydride	265/265/265	50/50/50 μL	10 sec	10 sec	10 sec
N-Methyl Imidazole	502/502/502	50/50/50 μL	10 sec	10 sec	10 sec
TCA	238/475/475	250/500/500 μL	15 sec	15 sec	15 sec
Iodine	6.8/6.8/6.8	80/80/80 μL	30 sec	30 sec	30 sec
Beaucage	34/51/51	80/120/120	100 sec	200 sec	200 sec
Acetonitrile	NA	1150/1150/1150 μL	NA	NA	NA

• Wait time does not include contact time during delivery.
• Tandem synthesis utilizes double coupling of linker molecule

[0263]

TABLE III

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
AF069669	SE8538	A
AF069671	SE7535	A
AF069673	SE8891	A
AF107771	UGSE8131	A
AF193275	97BL006 AF193275	A
AF361872	97TZ02 AF361872	A
AF361873	97TZ03 AF361873	A
AF413987	98UA0116 AF413987	A
AF004885	Q23-17	A1
AF069670	SE7253	A1
M62320	U455 U455A	A1
U51190	92UG037	A1
AF286237	94CY017.41	A2
AF286238	97CDKTB48	A2
A04321	IIIB LAI	B
AB078005	ARES2 AB078005	B
AF003887	WC001	B
AF003888	NL43WC001	B
AF004394	AD87 ADA	B
AF033819	HXB2-copy LAI	B
AF042100	MBC200	B
AF042101	MBC925	B
AF042102	MBC18 MBCC18	B
AF042103	MBCC54	B
AF042104	MBCC98	B
AF042105	MBCD36	B
AF042106	MBCC08R01 C18R01	B
AF049494	499JC16	B
AF049495	NC7	B
AF069140	DH12-3	B
AF070521	NL43E9 LAI IIIB/NY5	B
AF075719	MNTQ MNclone TQ	B
AF086817	TWCYS LM49	B
AF146728	VH	B
AF224507	WK	B
AF256204	S61I1 AF256204	B
AF256205	S61D15 AF256205	B
AF256206	S61G1 AF256206	B
AF256207	S61G7 AF256207	B
AF256208	S61I15 AF256208	B
AF256209	S61K1 AF256209	B
AF256210	S61K15 AF256210	B
AF256211	S61Dl1	B
AF286365	WR27	B
AJ006287	89SP061 89ES061	B
AJ271445	GB8 GB8-46R HIM271445	B
AX078307	BH10	B
AY037268	ARCH054	B
AY037269	ARMS008	B
AY037270	BOL 122	B
AY037274	ARMA173	B
AY037282	ARMA132	B
D10112	CAM1	B
D86068	MCK1	B
D86069	PM213	B
K02007	SF2 LAV2 ARV2	B
K02013	LAI BRU	B
K02083	PV22	B
K03455	HXB2 HXB2CG HXB2R LAI	B
L02317	BC BCSG3	B
L31963	TH475A LAI	B
M15654	BH102 BH10	B
M17449	MNCG MN	B
M17451	RF HAT3	B
M19921	NL43 pNL43 NL4-3	B
M26727	OYI, 397	B
M38429	JRCSF JR-CSF	B
M38431	NY5CG	B
M93258	YU2 YU2X	B
M93259	YU10	B

TABLE III-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
NC_001802	HXB2R	B
U12055	LW123	B
U21135	WEAU160 GHOSH	B
U23487	contaminant MANC	B
U26546	WR27	B
U26942	NL4-3 LAI/NY5 pNL43 NL43	B
U34603	H0320-2A12 ACH3202A12	B
U34604	3202A21 ACH3202A21	B
U37270	C18MBC	B
U39362	P896 89.6	B
U43096	D31	B
U43141	HAN	B
U63632	JRFL JR-FL	B
U69584	85WCIPR54	B
U69585	WCIPR854	B
U69586	WCIPR8546	B
U69587	WCIPR8552	B
U69588	WCIPR855	B
U69589	WCIPR9011	B
U69590	WCIPR9012	B
U69591	WCIPR9018	B
U69592	WCIPR9031	B
U69593	WCIPR9032	B
U71182	RL42	B
X01762	REHTLV3 LAI IIIB	B
Z11530	F12CG	B

[0264]

TABLE IV

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
AB032740	95TNIH022	01_AE
AB032741	95TNIH047	01_AE
AB052995	93JPNH1	01_AE
AB070352	NH25 93JPNH25T 93JP-NH2.5T	01_AE
AB070353	NH2 93JPNH2ENV	01_AE
AF164485	93TH9021	01_AE
AF197338	93TH057	01_AE
AF197339	93TH065	01_AE
AF197340	90CF11697 AF197340	01_AE
AF197341	90CF4071 AF197341	01_AE
AF259954	CM235-2	01_AE
AF259955	CM235-4	01_AE
AY008714	97CNGX2F 97CNGX-2F	01_AE
AY008718	97CNGX11F	01_AE
U51188	90CF402 90CR402 CAR-E 4002	01_AE
U51189	93TH253	01_AE
U54771	CM240	01_AE
AF362994	NP1623	01B
AY082968	TH1326 AY082968	01B
AJ404325	97DCKTB49 97CDKTB49 HIM404325	01GHJKU
AB049811	97GHAG1 AB049811	02_AG
AB052867	AB052867	02_AG
AF063223	DJ263	02_AG
AF063224	DJ264	02_AG
AF107770	SE7812	02_AG
AF184155	G829	02_AG
AF377954	CM52885 AF377954	02_AG
AF377955	CM53658 AF377955	02_AG
AJ251056	MP1211 98SE-MP1211	02_AG
AJ251057	MP1213 98SEMP1213 HIM251057	02_AG
AJ286133	97CM-MP807	02_AG
L39106	IBNG	02_AG
AF193276	KAL153-2	03_AB
AF193277	RU98001 98RU001	03_AB

TABLE IV-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
AF414006	98BY10443 AF414006	03-AB
AF049337	94CY032-3 CY032.3	04_cpx
AF119819	97PVMY GR84	04_cpx
AF119820	97PVCH GR11	04_cpx
AF076998	VI961	05_DF
AF193253	VI1310 AF193253	05_DF
AF064699	BFP90	06_cpx
AJ245481	95ML84	06_cpx
AJ288981	97SE1078	06_cpx
AJ288982	95ML127	06_cpx
AF286226	97CN001 054	07_BC
AF286230	98CN009	07_BC
AX149647	C54A C54	07_BC
AX149672	C54D AX149672	07_BC
AX149771	CN54b	07_BC
AX149898	C54C	07_BC
AF286229	98CN006	08_BC
AY008715	97CNGX6F	08_BC
AY008716	97CNGX7F	08_BC
AY008717	97CNGX9F	08_BC
AF289548	96TZBF061	10_CD
AF289549	96TZBF071	10_CD
AF289550	96TZBF110	10_CD
AF179368	GR17	11_cpx
AJ291718	MP818	11_cpx
AJ291719	MP1298	11_cpx
AJ291720	MP1307	11_cpx
AF385934	URTR23	12_BF
AF385935	URTR35	12_BF
AF385936	ARMA159	12_BF
AF408629	A32879 AF408629	12_BF
AF408630	A32989 AF408630	12_BF
AY037279	ARMA185	12_BF
AF423756	X397 AF423756	14_BG
AF423757	X421 AF423757	14_BG
AF423758	X475 AF423758	14_BG
AF423759	X477 AF423759	14_BG
AF450096	X605 AF450096	14_BG
AF450097	X623 AF450097	14_BG
AF069669	SE8538	A
AF069671	SE7535	A
AF069673	SE8891	A
AF107771	UGSE8131	A
AF193275	97BL006 AF193275	A
AF361872	97TZ02 AF361872	A
AF361873	97TZ03 AF361873	A
AF413987	98UA0116 AF413987	A
AF004885	Q23-17	A1
AF069670	SE7253	A1
M62320	U455 U455A	A1
U51190	92UG037	A1
AF286237	94CY017.41	A2
AF286238	97CDKTB48	A2
U86780	ZAM184	A2C
AF286239	97KR004	A2D
AF316544	97CDKP58	A2G
AF067156	95IN21301	AC
AF071474	SE9488	AC
AF361871	97TZ01 AF361871	AC
AF361876	97TZ06 AF361876	AC
AF361878	97TZ08 AF361878	AC
AF361879	97TZ09 AF361879	AC
U88823	92RW009	AC
AF075702	SE8603	ACD
AJ276595	VI1035	ACG
AF071473	SE7108	AD
AF075701	SE6954	AD
AJ237565	97NOGIL3	ADHK
X04415	MAL MALCG	ADK
AF377959	CM53379 AF377959	AFGHJU
AF377957	CM53392 AF377957	AG
AJ276596	VI1197	AG

TABLE IV-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
U88825	92NG003	AG
AF076474	VI354	AGHU
AF192135	BW2117	AGJ
AJ293865	B76 HIM293865	AGJ
AF069672	SE6594	AU
A04321	IIIB LAI	B
AB078005	ARES2 AB078005	B
AF003887	WC001	B
AF003888	NL43WC001	B
AF004394	AD87 ADA	B
AF033819	HXB2-copy LAI	B
AF042100	MBC200	B
AF042101	MBC925	B
AF042102	MBC18 MBCC18	B
AF042103	MBCC54	B
AF042104	MBCC98	B
AF042105	MBCD36	B
AF042106	MBCC18R01 C18R01	B
AF049494	499JC16	B
AF049495	NC7	B
AF069140	DH12-3	B
AF070521	NL43E9 LAI IIIB/NY5	B
AF075719	MNTQ MNcloneTQ	B
AF086817	TWCYS LM49	B
AF146728	VH	B
AF224507	WK	B
AF256204	S61I1 AF256204	B
AF256205	S61D15 AF256205	B
AF256206	S61G1 AF256206	B
AF256207	S61G7 AF256207	B
AF256208	S61I15 AF256208	B
AF256209	S61K1 AF256209	B
AF256210	S61K15 AF256210	B
AF256211	S61D1	B
AF286365	WR27	B
AJ006287	89SP061 89ES061	B
AJ271445	GB8 GB8-46R HIM271445	B
AX078307	BH10	B
AY037268	ARCH054	B
AY037269	ARMS008	B
AY037270	BOL122	B
AY037274	ARMA173	B
AY037282	ARMA132	B
D10112	CAM1	B
D86068	MCK1	B
D86069	PM213	B
K02007	SF2 LAV2 ARV2	B
K02013	LAI BRU	B
K02083	PV22	B
K03455	HXB2 HXB2CG HXB2R LAI	B
L02317	BC BCSG3	B
L31963	TH475A LAI	B
M15654	BH102 BH10	B
M17449	MNCG MN	B
M17451	RF HAT3	B
M19921	NL43 pNL43 NL4-3	B
M26727	OYI, 397	B
M38429	JRCSF JR-CSF	B
M38431	NY5CG	B
M93258	YU2 YU2X	B
M93259	YU10	B
NC_001802	HXB2R	B
U12055	LW123	B
U21135	WEAU160 GHOSH	B
U23487	contaminant MANC	B
U26546	WR27	B
U26942	NL4-3 LAI/NY5 pNL43 NL43	B
U34603	H0320-2A12 ACH3202A12	B
U34604	3202A21 ACH3202A21	B
U37270	C18MBC	B
U39362	P896 89.6	B
U43096	D31	B

TABLE IV-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
U43141	HAN	B
U63632	JRFL JR-FL	B
U69584	85WCIPR54	B
U69585	WCIPR854	B
U69586	WCIPR8546	B
U69587	WCIPR8552	B
U69588	WCIPR855	B
U69589	WCIPR9011	B
U69590	WCIPR9012	B
U69591	WCIPR9018	B
U69592	WCIPR9031	B
U69593	WCIPR9032	B
U71182	RL42	B
X01762	REHTLV3 LAI IIIB	B
Z11530	F12CG	B
AF332867	A027 AF332867	BF
AF408626	A025 AF408626	BF
AF408627	A047 AF408627	BF
AF408628	A063 AF408628	BF
AF408631	A050 AF408631	BF
AE408632	A32878 AF408632	BF
AY037266	ARCH014	BF
AY037267	ARCH003	BF
AY037271	BOL137	BF
AY037272	URTR17	BF
AY037273	ARMA062	BF
AY037275	ARMA036	BF
AY037276	ARMA070	BF
AY037277	ARMA037	BF
AY037278	ARMA006	BF
AY037280	ARMA097	BF
AY037281	ARMA038	BF
AY037283	ARMA029	BF
AF005495	93BR029.4	BF1
AF423755	X254 AF423755	BG
AB023804	93IN101	C
AF067154	93IN999 301999	C
AF067155	95IN21068	C
AF067157	93IN904 301904	C
AF067158	93IN905 301905	C
AF067159	94IN11246	C
AF110959	96BW01B03 96BW01B03	C
AF110960	96BW01B21	C
AF110961	96BW01B22	C
AF110962	96BW0402	C
AF110963	96BW0407	C
AF110964	96BW0408	C
AF110965	96BW0409	C
AF110966	96BW0410	C
AF110967	96BW0502	C
AF110968	96BW0504	C
AF110969	96BW1104	C
AF110970	96BW1106	C
AF110971	96BW11B01	C
AF110972	96BW1210	C
AF110973	96BW15B03	C
AF110974	96BW15C02	C
AF110975	96BW15C05	C
AF110976	96BW16B01	C
AF110977	96BW16D14	C
AF110978	96BW1626	C
AF110979	96BW17A09	C
AF110980	96BW17B03	C
AF110981	96BW17B05	C
AF286223	94IN476	C
AF286224	96ZM651	C
AF286225	96ZM751	C
AF286227	97ZA012	C
AF286228	98BR004	C
AF286231	98IN012	C
AF286232	98IN022	C
AF286233	98IS002	C

TABLE IV-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
AF286234	98TZ013	C
AF286235	98TZ017	C
AF290027	96BW06H51 96BW06-H51	C
AF290028	96BW06J4	C
AF290029	96BW06J7 AF290029	C
AF290030	96BW06K18 AF290030	C
AF321523	MJ4	C
AF361874	97TZ04 AF361874	C
AF361875	97TZ05 AF361875	C
AF443074	96BWMO15	C
AF443075	96BWM032 AF443075	C
AF443076	98BWMC122 AF443076	C
AF443077	98BWMC134 AF443077	C
AF443078	98BWMC14A3 AF443078	C
AF443079	988WMO1410 AF443079	C
AF443080	98BWMO18D5 AF443080	C
AF443081	98BWMO36A5 AF443081	C
AF443082	98BWMO37D5 AF443082	C
AF443083	99BW393212 AF443083	C
AF443084	99BW46424 AF443084	C
AF443085	99BW47458 AF443085	C
AF443086	99BW47547 AF443086	C
AF443087	99BWMC168 AF443087	C
AF443088	00BW07621 AF443088	C
AF443089	00BW076820 AF443089	C
AF443090	00BW087421 AF443090	C
AF443091	00BW147127 AF443091	C
AF443092	00BW16162 AF443092	C
AF443093	00BW1686. 00BW16868 AF443093	C
AF443094	00BW17593 AF443094	C
AF443095	00BW17732 AF443095	C
AF443096	00BW17835 AF443096	C
AF443097	00BW17956 AF443097	C
AF443098	00BW18113 AF443098	C
AF443099	00BW18595 AF443099	C
AF443100	00BW18802 AF443100	C
AF443101	00BW192113 AF443101	C
AF443102	00BW20361 AF443102	C
AF443103	00BW20636 AF443103	C
AF443104	00BW20872 AF443104	C
AF443105	00BW2127214 AF443105	C
AF443106	00BW21283 AF443106	C
AF443107	00BW22767 AF443107	C
AF443108	00BW38193 AF443108	C
AF443109	00BW38428 AF443109	C
AF443110	00BW38713 AF443110	C
AF443111	00BW38769	C
AF443112	00BW38868	C
AF443113	00BW38916	C
AF443114	00BW39702	C
AF443115	00BW50311	C
AY043173	DU151 AY043173	C
AY043174	DU179 AY043174	C
AY043175	DU422 AY043175	C
AY043176	CTSC2 AY043176	C
U46016	ETH2220 02220	C
U52953	92BR025	C
AF361877	97TZ07 AF361877	CD
AY074891	00BWMO351 AY074891	CD
AF133821	MB2059	D
AJ320484	HIM320484	D
K03454	ELI	D
M22639	Z2Z6 Z2 CDC-Z34	D
M27323	NDK	D
U88822	84ZR085	D
U88824	94UG1141	D
AF005494	93BR020.1	F1
AF075703	FIN9363	F1
AF077336	VI850	F1
AJ249238	MP411 96FRMP411	F1
AF377956	CM53657 AF377956	F2
AJ249236	MP255 95CMMP255	F2

TABLE IV-continued

HUMAN HIV-1 SEQUENCES

Accession	Name	Subtype
AJ249237	MP257 95CM-MP257C	F2
AF076475	VI1126	F2KU
AF061640	HH8793-1.1	G
AF061641	HH8793-12.1	G
AF061642	SE6165 G6165	G
AF084936	DRCBL	G
AF423760	X558 AF423760	G
AF450098	X138 AF450098	G
U88826	92NG083 JV10832	G
AF005496	90CF056 90CR056	H
AF190127	VI991	H
AF190128	VI997	H
AF082394	SE7887 SE92809	J
AF082395	SE7022 SE9173	J
AJ249235	EQTB11C 97ZR-EQTB11C	K
AJ249239	MP535 96CM-MP535C	K
AJ239083	97CA-MP645M/O	MO
AJ006022	YBF30	N
AJ271370	YBF106	N
AF407418	VAU AF407418	O
AF407419	VAU AF407419	O
AJ302646	SEMP1299 HIM302646	O
AJ302647	SEMP1300 HIM302647	O
L20571	MVP5180	O
L20587	ANT70	O
NC_002787	SEMP1299 NC_002787	O
AF286236	83CD003 Z3 AF286236	U
AF457101	90CD121E12 AF457101	U
AY046058	GR303 99GR303 AY046058	U

What we claim is:

1. A short interfering RNA (siRNA) molecule that down regulates expression of a human immunodeficiency virus (HIV) gene by RNA interference.

2. The siRNA molecule of claim 1, wherein said siRNA molecule is adapted for use to treat HIV infection or acquired immunodeficiency syndrome (AIDS).

3. The siRNA molecule of claim 1, wherein said siRNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises sequence complementary to a HIV RNA sequence and the sense region comprises sequence complementary to the antisense region.

4. The siRNA molecule of claim 3, wherein said siRNA molecule is assembled from two nucleic acid fragments wherein one fragment comprises the sense region and the second fragment comprises the antisense region of said siRNA molecule.

5. The siRNA molecule of claim 4, wherein said sense region and antisense region are covalently connected via a linker molecule.

6. The siRNA molecule of claim 5, wherein said linker molecule is a polynucleotide linker.

7. The siRNA molecule of claim 5, wherein said linker molecule is a non-nucleotide linker.

8. The siRNA molecule of claim 3, wherein said antisense region comprises sequence complementary to sequence having any of SEQ ID NOs. 1-738.

9. The siRNA molecule of claim 3, wherein said antisense region comprises sequence having any of SEQ ID NOs. 739-1476.

10. The siRNA molecule of claim 3, wherein said sense region comprises sequence having any of SEQ ID NOs. 1-738.

11. The siRNA molecule of claim 3, wherein said sense region comprises a 3'-terminal overhang and said antisense region comprises a 3'-terminal overhang.

12. The siRNA molecule of claim 11, wherein said 3'-terminal overhangs each comprise about 2 nucleotides.

13. The siRNA molecule of claim 11, wherein said antisense region 3'-terminal nucleotide overhang is complementary to a HIV RNA.

14. The siRNA molecule of claim 3, wherein said sense region comprises one or more 2'-O-methyl modified pyrimidine nucleotides.

15. The siRNA molecule of claim 3, wherein said sense region comprises a terminal cap moiety at the 5'-end, 3'-end, or both 5' and 3' ends of said sense region.

16. The siRNA molecule of claim 3, wherein said antisense region comprises one or more 2'-deoxy-2'-fluoro modified pyrimidine nucleotides.

17. The siRNA molecule of claim 3, wherein said antisense region comprises a phosphorothioate internucleotide linkage at the 3' end of said antisense region.

18. The siRNA molecule of claim 3, wherein said antisense region comprises between about one and about five phosphorothioate internucleotide linkages at the 5' end of said antisense region.

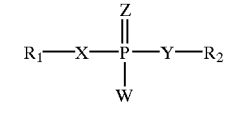
19. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise ribonucleotides that are chemically modified at a nucleic acid sugar, base, or backbone.

20. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise deoxyribonucleotides that are chemically modified at a nucleic acid sugar, base, or backbone.

21. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise one or more universal base ribonucleotides.

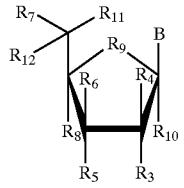
22. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise one or more acyclic nucleotides.

23. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise nucleotides comprising internucleotide linkages having Formula I:



wherein each R1 and R2 is independently any nucleotide, non-nucleotide, or polynucleotide which can be naturally occurring or chemically modified, each X and Y is independently O, S, N, alkyl, or substituted alkyl, each Z and W is independently O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl, and wherein W, X, Y and Z are not all O.

24. The siRNA molecule of claim 11, wherein said 3'-terminal nucleotide overhangs comprise nucleotides or non-nucleotides having Formula II:



wherein each R3, R4, R5, R6, R7, R8, R10, R11 and R12 is independently H, OH, alkyl, substituted alkyl, alkaryl or aralkyl, F, Cl, Br, CN, CF3, OCF3, OCN, O-alkyl, S-alkyl, N-alkyl, O-alkenyl, S-alkenyl, N-alkenyl, SO-alkyl, alkyl-OSH, alkyl-OH, O-alkyl-OH, O-alkyl-SH, S-alkyl-OH, S-alkyl-SH, alkyl-S-alkyl, alkyl-O-alkyl, ONO2, NO2, N3, NH2, aminoalkyl, aminoacid, aminoacyl, ONH2, O-aminoalkyl, O-aminoacid, O-aminoacyl, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalklylamino, substituted silyl, or group having Formula I; R9 is O, S, CH2, S═O, CHF, or CF2, and B is a nucleosidic base or any other non-naturally occurring base that can be complementary or non-complementary to HIV RNA or a non-nucleosidic base or any other non-naturally occurring universal base that can be complementary or non-complementary to HIV RNA.

25. An expression vector comprising a nucleic acid sequence encoding at least one siRNA molecule of claim 1 in a manner that allows expression of the nucleic acid molecule.

26. A mammalian cell comprising an expression vector of claim 25.

27. The mammalian cell of claim 26, wherein said mammalian cell is a human cell.

28. The expression vector of claim 25, wherein said siRNA molecule comprises a sense region and an antisense region and wherein said antisense region comprises sequence complementary to a HIV RNA sequence and the sense region comprises sequence complementary to the antisense region.

29. The expression vector of claim 28, wherein said siRNA molecule comprises two distinct strands having complementarity sense and antisense regions.

30. The expression vector of claim 28, wherein said siRNA molecule comprises a single strand having complementary sense and antisense regions.

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